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# TRICHLOROACETIC ACID AS AN EXPOSURE BIOMARKER FOR DISINFECTION BY-PRODUCTS IN A CONTROLLED EXPOSURE STUDY

by

**ERIN LEANE BADER**



A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Trichloroacetic Acid as an Exposure Biomarker for Disinfection By-products in a Controlled Exposure Study** submitted by **Erin Leane Bader** in partial fulfillment of the requirements for the degree of **Masters of Science in Medical Sciences – Public Health Sciences**.

To my family.

Thank you for your unwavering encouragement and support of my education,
and for being a part of and playing a role in the successes I achieve.

## ABSTRACT

The ability of epidemiologic studies to show a causal relationship between exposure to disinfection by-products (DBPs) and adverse reproductive outcomes has been hindered by inaccurate assessment of individual exposure to DBPs. Validating trichloroacetic acid (TCAA), one DBP, as an exposure biomarker when measured in urine has shown promise as a means of providing individual-level exposure assessment in epidemiologic studies. For TCAA to be a valid biomarker of DBP exposure it must be sufficiently persistent in the human body and levels of TCAA in urine need to correlate with levels of TCAA in drinking water. By determining urinary elimination half-life for TCAA, it was found to be persistent enough in the human body to facilitate its use as an exposure biomarker. First morning urine (FMU) samples were determined to be representative of total daily TCAA excretion, and half-life determination was the same whether FMU or average daily urine was used for calculation. Both within and among individuals, variability in ingestion and excretion of TCAA was observed despite management of TCAA intake sources and volumes. This variability was attributed to differences in water ingestion volumes and elimination kinetics. Higher levels of TCAA ingestion correlated with higher levels of TCAA excretion, indicating improved confidence in using TCAA in urine as an exposure biomarker for DBPs.

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# List of Abbreviations

| | |
|---|---|
| BDCM | Bromodichloromethane |
| CDBM | Chlorodibromomethane |
| CH | Chloral hydrate |
| CHM | Chlorinating municipality |
| CI | Confidence interval |
| CNS | Central nervous system |
| DBP(s) | Disinfection by-product(s) |
| DCAA | Dichloroacetic acid |
| DMKL | Dynacare Kasper Medical Laboratory |
| ECD | Electron capture detection/detector |
| EPA | Environmental Protection Agency |
| FMU | First morning urine |
| GC | Gas chromatography/chromatograph |
| HAA(s) | Haloacetic acid(s) |
| ILSI | International Life Sciences Institute |
| IUGR | Intrauterine growth retardation |
| LBW | Low birthweight |
| MDL | Method detection limit |
| MTBE | Methyl tert-butyl ether |
| MX | 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone |
| NCHM | Non-chlorinating municipality |
| OR | Odds ratio |
| PDMS | Polydimethyl siloxane |
| RR | Relative risk |
| SD | Standard deviation |
| SGA | Small for gestational age |
| SPME | Solid phase microextraction |
| TCAA | Trichloroacetic acid |
| TCE | Trichloroethylene |
| THM(s) | Trihalomethane(s) |
| TLBW | Term low birthweight |
| TTHM(s) | Total trihalomethane(s) |
| USEPA | United States Environmental Protection Agency |
| VLBW | Very low birthweight |

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# I GENERAL INTRODUCTION

Water, according to ancient philosophers, was the first element, "the mother of all things" (Walker 1983). In parallel, the contemporary evolutionary perspective suggests water is where organic life began (Campbell 1993) and, biologically, is required for the sustenance of life. Though water is a life-giving force, it is also capable of carrying pathogens that cause death and disease. For this reason, disinfection of drinking water is an important and necessary treatment step for public water supplies.

The practice of disinfecting drinking water with chlorine was widely introduced just after the turn of the twentieth century. As a result, the public health impacts associated with microbially contaminated drinking water have been largely eliminated in the developed world where disinfection methods are sufficient and reliable. Table I-1 lists some of the acute waterborne diseases whose incidence can be reduced or prevented through appropriate drinking water disinfection. Contrary to the situation in the developed world, in developing countries where water disinfection is neither reliable nor sufficient, childhood diarrheal disease alone accounts for over 2 million deaths annually (World Bank 1993). Drinking water disinfection is clearly of importance to public health.

Table I-1. Waterborne diseases prevented or reduced by adequate water disinfection.

| **Bacterial** | **Viral** | **Parasitic** |
|---|---|---|
| Typhoid fever | Hepatitis | Amebiasis |
| Paratyphoid | Rotaviral diarrhea | Giardiasis |
| Childhood bacterial diarrhea | Norwalk agent diarrhea | Cryptosporidiasis |
| *Cholera* | | |
| *Salmonella* | | |
| *Shigella* | | |
| *Yersinia* | | |
| *Campylobacter* | | |
| Hemorrhagic *E. coli.* | | |

The benefits of water disinfection remained unchallenged until the discovery that chlorination of drinking water leads to the formation of disinfection by-products (DBPs). In 1974 it was reported that trihalomethanes (THMs), a class of chemical compounds that includes chloroform, bromoform, chlorodibromomethane (CDBM), and bromodichloromethane (BDCM), were formed following chlorination of public water

supplies (Bellar et al. 1974; Rook 1974). This discovery coincided with the findings that chloroform caused cancer in rodents (National Cancer Institute 1976) and that chloroform is ubiquitous in chlorinated drinking water (Symons 1975), generating concern over the potential health effects of chronic exposure to DBPs in drinking water. As a result, many more DBPs have since been identified including classes such as haloacetic acids (HAAs), haloacetonitriles, haloketones, aldehydes, halopicrins, and cyanogen halides and individual compounds such as chloral hydrate, 2,4,6-trichlorophenol, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) (Richardson 1998) and many more compounds yet to be identified.

Available toxicologic evidence from animal studies suggests a number of the above DBPs are carcinogenic, mutagenic, and/or teratogenic (Bull 1985; EPA/ILSI 1993; Austin et al. 1996; Parrish et al. 1996; James et al. 1997). Data from toxicologic studies, however, is produced by administering individual DBPs at doses that are often several orders of magnitude higher than those mixtures of by-products that occur in drinking water. This intrinsic limitation prevents determining causality for relevant doses from toxicologic data alone. Therefore, epidemiologic studies with humans exposed to DBPs at drinking water levels are required to support the toxicologic information.

Although initially focussed on cancer, epidemiologic studies examining the relationship between DBPs and health have more recently concerned adverse reproductive outcomes. Epidemiologic studies have found associations between exposure to by-products of chlorination and adverse birth outcomes including small for gestational age, stillbirth, spontaneous abortion, low birth weight, and congenital anomalies (Bove et al. 1995; Savitz et al. 1995; Gallagher et al. 1998; Swan and Waller 1998; Waller et al. 1998; Dodds et al. 1999; Magnus et al. 1999; King et al. 2000; Yang et al. 2000). As a group, these studies suggest a relationship between exposure to DBPs and adverse reproductive outcomes, however, the evidence from them is insufficient to support causality.

Demonstrating causal associations between suspected agents and human health outcomes requires the epidemiologic method to obtain individual data on both adverse outcomes and exposure to the putative agent. In the aforementioned studies individual outcome

data has often been reliably ascertained, however, individual exposure evidence for these studies has not been accurately quantified. Some studies have used the over-simplified method of assessing exposure based on whether areas in which individuals lived did or did not receive chlorinated water. Other exposure methods have used DBP concentrations measured at treatment plants or in samples of tap water. Improvements to using water quality data have been achieved by assessing water consumption and usage with questionnaires and combining these exposure methods with individual water quality data. However, none of these studies has used true individual monitoring for exposure assessment. As a consequence, the potential for exposure misclassification in these studies is high.

Misclassification of exposure, if it is random (unrelated to exposure or outcome), generally has the effect of biasing results of epidemiologic studies towards the null but in specialized circumstances can also bias results away from the null (Reif et al. 2000). Such a bias means the true measure of risk for adverse reproductive effects of exposure to DBPs, if exposure was properly assessed and the agent is causal, could be higher or lower than the relative risks reported in the current literature. Thus, there is a need to provide adequate means of assessing individual exposure to DBPs because any causal relationship between DBPs and adverse reproductive outcomes, even if associated with a small relative risk, is important to public health because of the wide population exposure to some level of DBPs in chlorinated drinking water.

The need to refine epidemiologic methods to improve understanding of any causal relationship between exposure to DBPs and adverse reproductive outcomes can potentially be realized by using exposure biomarkers. Biomarkers of exposure are parent compounds or their metabolites that show a quantifiable relationship to the amount of compound to which an individual was exposed. Thus, if DBPs or their respective metabolites can be measured in human biological media, and these measurements correlate to levels of DBPs in drinking water, there is potential for biomarkers to provide individual exposure assessment for use in epidemiologic studies.

The measurements and correlation described above cannot be utilized in future epidemiologic studies concerning DBPs in drinking water unless exposure biomarkers are available, feasible, and validated. This thesis explores the validity and feasibility of using trichloroacetic acid (TCAA), an individual DBP, as an exposure biomarker for by-products of disinfection in a controlled human exposure study. In this chapter, I further discuss the formation and properties of DBPs, provide extensive review of the current epidemiologic literature on DBPs and adverse reproductive outcomes, describe the rationale for and the desirable characteristics of using exposure biomarkers, and outline the specific methodology used to measure TCAA in this thesis. Chapter II characterizes the persistence of TCAA in the human body using measurements of elimination half-life and explores different methodologies for increasing the feasibility of using TCAA as an exposure biomarker in epidemiologic studies. Chapter III describes the relationship between ingested and excreted TCAA and examines the intra- and inter-individual variations in TCAA ingestion and excretion. Chapter IV provides conclusions.

### *Formation of DBPs*

Chlorine, in water, exists as hypochlorous acid and hypochlorite ion (Equation 1). Hypochlorous acid is responsible for the inactivation of pathogens in disinfected water via oxidation reactions, however, hypochlorous acid also undergoes addition and substitution reactions with naturally occurring organic matter in source water to form DBPs (Bellar et al. 1974; Rook 1974). The organic matter in source water is a collection of humic substances formed as a consequence of the natural degradation of soil and vegetative matter. A number of factors influence the extent of DBP formation in finished waters including pH, temperature, type and amount of precursors, chlorine concentration, and chlorine contact time (Singer 1993). Generally, drinking water that originates from surface water yields a higher concentration of DBPs than drinking water from ground water sources, because surface water contains a higher level of organic precursors. Furthermore, there is a seasonal variation in DBP formation, where DBP concentrations are highest in summer and early fall, attributed to the increased temperature and level of dissolved organic matter present in the water during these seasons (Singer 1993).

$$Cl_{2(g)} + H_2O \leftrightarrow HOCl + HCl; HOCl \leftrightarrow H^+ + OCl^- \quad (1)$$

It is important to note that chlorine is not the only disinfectant available to treat water intended for human consumption. It is, however, the most widely used disinfectant (Singer 1993). Other disinfectants, although they do not create the same type or amount of by-products as chlorine, do create by-products in their own right. Consequently, there is no zero-risk disinfectant. Little toxicologic and epidemiologic data are available for the by-products of alternative disinfectants because chlorine has been the favored chemical for water disinfection in North America. As such, chlorination by-products will be the focus of this thesis.

The most prevalent DBPs in drinking water, on a mass-basis, are the THMs followed by the HAAs. The primary physiochemical difference between the THMs and the HAAs is that THMs are volatile and the HAAs are not. As mentioned previously, the THMs are a group of compounds that includes chloroform, BDCM, CDBM, and bromoform. Chloroform is the most prevalent THM in most chlorinated waters. The prevalence of the brominated THMs depends on the bromide content of the source water (Singer 1993). Of the HAAs, TCAA and dichloroacetic acid (DCAA) dominate in chlorinated waters (Krasner et al. 1989).

***Exposure Complexities for DBPs***

Humans are exposed to DBPs by three routes – ingestion, inhalation, and dermal absorption. Common household uses of chlorinated water that expose individuals to DBPs include direct consumption of water, food preparation using chlorinated tap water (ingestion exposure); bathing, showering, washing, swimming, hot-tub use (dermal and inhalation exposures); and running dishwashers, washing machines, and humidifiers (inhalation exposure) (Weisel et al. 1999). The contribution of each exposure route to the overall exposure to DBPs from chlorinated water depends on the respective physiochemical properties of specific DBPs and the water practices of the individual. Exposure to DBPs is also influenced by seasonal variation and distribution system characteristics as described previously.

In terms of bioavailability, DBP concentrations in chlorinated water are distinguished as the *external exposure*, that amount of compound that is absorbed (ingested, inhaled, or absorbed through the skin) is defined as the *external dose*, and the dose of DBPs that reaches the systemic circulation is the *internal dose* (Hrudey et al. 1996). Because the THMs are volatile and non-polar compounds, inhalation (volatile) and dermal absorption (non-polar) contribute most significantly to the external dose of THMs to the body. Elevated levels of individual THMs have been measured in exhaled breath after both inhalation and dermal exposures during showering and dermal exposure via bathing (Weisel and Jo 1996). Although ingestion contributes to the external dose of THMs, first pass metabolism of ingested THMs in the liver results in THMs being metabolized before entering the bloodstream (Weisel and Jo 1996). Therefore, ingestion does not contribute to the internal dose of THMs received. Whereas the THMs are volatile, the HAAs are nonvolatile and, therefore, are received in the body primarily through ingestion. In addition to being nonvolatile, the HAAs are polar and ionic compounds. Because the skin is relatively impermeable to polar and ionic compounds, dermal absorption during routine household uses is a minor contributor to the total exposure to HAAs (<1%). However, dermal absorption of HAAs from swimming pools can increase total exposure to HAAs by 10 – 20 percent because of the high concentrations of HAAs in chlorinated pool water (Kim and Weisel 1998).

Variation in water practices among individuals also influences exposure to DBPs. Reductions in exposure to DBPs are experienced by those individuals who consume bottled water and use home filtration systems (Reif et al. 2000). Similarly reductions in exposure to volatile DBPs are received when heated water is consumed or cold tap water is left to stand before consumption (Kim et al. 1999). Conversely, increased levels of DBPs are received when chlorinated tap water is used for prepared beverages due to the interaction of free chlorine with organic matter in the form of foodstuffs (Balko et al. 2001). Variability in showering, bathing, and washing habits and the characteristics of the enclosures in which these activities are performed also influences the amount of DBPs to which an individual is exposed (Reif et al. 2000).

*Capability of Epidemiology to Demonstrate Causation*

Before interpreting the various epidemiologic studies investigating the relationship between DBPs and adverse reproductive outcomes it is necessary to discuss the power of epidemiology to detect associations. All epidemiologic studies are designed to show if there is a correlation between an exposure and an outcome. The basic rationale for categorizing exposure and outcome for epidemiologic studies is shown in Table I-2. Exposure categories are indicated in the horizontal rows and the vertical columns show disease categories. In the simplest case of categorical rather than continuous exposure classification these variables are used to compute whether there is an association between the exposure and outcome of interest.

Table I-2. Rationale for exposure and disease categorization for epidemiologic studies.

| | **Disease** | **No Disease** |
|---|---|---|
| **Exposure** | a | b |
| **No Exposure** | c | d |

Source: Hennekens and Buring 1987.

The strength of the association between exposure and outcome for an epidemiologic study depends on the magnitude of its relative risk (RR). The RR is defined as "the ratio of incidence of disease in the exposed group divided by the corresponding incidence of disease in the non-exposed group" (Hennekens and Buring 1987). Using the variables indicated in Table I-2, the RR is calculated according to the following equation:

$$RR = \frac{a/(a+b)}{c/(c+d)} = \frac{\text{disease (exposed)/total exposed}}{\text{disease (not exposed)/total not exposed}} \qquad (2)$$

A RR of 1.0 indicates that there is no association observed between the exposure and the disease because the incidence of disease in the exposed group is equal to the incidence of the disease in the non-exposed group. If the value of the RR is greater than 1.0, it indicates an increased risk of disease among those exposed to the suspected cause as compared to those who are not exposed. Correspondingly, a RR less than 1.0 indicates that there is a decreased risk of disease among those who are exposed compared to those

who are not exposed, suggesting a protective value of being exposed to the suspected causal agent.

Usually included with measures of relative risk are confidence intervals. Confidence intervals indicate the range within which the true measure of RR lies with a certain amount of statistical uncertainty (Hennekens and Buring 1987). The 95-percent confidence interval is the most commonly used significance level and means one can be 95 percent confident that the true level of risk lies within the range of RR values indicated by the confidence interval. Larger confidence intervals (for the same confidence level) indicate less certainty that the RR measured is the true RR. Wide confidence intervals usually indicate that the sample from which the RR was measured is small. Furthermore, confidence intervals that include the RR value of 1.0 are not statistically significant (at the specified level of confidence) because it cannot be determined if there is truly an increased or reduced relative risk or if the values observed occurred by chance alone.

The above explanation indicates the ability of the epidemiologic method to show a correlation between an exposure and an outcome. Conclusions about causation, however, require adequately controlling bias and confounding as explanations for the findings and require the epidemiologic method to obtain individual data on both exposure and outcome. The ability to limit bias and confounding, and thus contribute evidence of causation (Hill 1965), is largely a consequence of study design. Two distinct classes of epidemiologic studies, experimental and observational, exist. Table I-3 summarizes these two classes of studies and includes sub-types of each class, alternative name, and unit of study.

Table I-3. Types of epidemiologic studies.

| Type of Study | Unit of Study | Capability |
|---|---|---|
| 1. Experimental studies | Individuals | Hypothesis testing |
| 2. Observational studies | | |
| a. Ecological studies | Populations | Hypothesis generating |
| b. Cross-sectional studies | Individuals | Hypothesis generating |
| c. Case-control studies | Individuals | Hypothesis testing |
| d. Cohort studies | Individuals | Hypothesis testing |

*Experimental Studies*

Experimental studies, commonly known as clinical trials when used for drugs or medical interventions, provide the greatest control over bias and confounding because individuals are randomly allocated into exposure groups by study personnel. Individuals are then followed for the occurrence of health outcomes (Hennekens and Buring 1987). As a controlled study, an experimental design would provide the strongest epidemiologic evidence towards judging whether an observed association between exposure to DBPs and adverse reproductive outcomes is one of cause and effect. However, because these studies require deliberately exposing people to compounds that are suspected of being harmful, experimental studies are generally not feasible for investigating the relationship between DBPs and adverse reproductive outcomes. Ethical constraints could be overcome by intervening to reduce exposures among one group relative to their normal exposure. However, the feasibility of doing this in a random manner for reproductive outcomes for a cohort of sufficient size is limited. An additional requirement of blinding the participants and the investigators to the exposure status does not seem possible.

*Observational Studies*

In observational studies, study personnel do not allocate exposure; they simple observe who is 'naturally' exposed or not exposed and who has or does not have the outcome of interest. Therefore, observational studies are applicable to the study of adverse reproductive effects from exposure to DBPs. There are several levels of observational study (Table I-3).

Ecological studies, also called correlational studies, use population-based measures of exposure and disease outcome. Because this study design does not obtain individual level information on exposure and disease, it is limited to being hypothesis-generating. In population-based measures, whether the individuals who were exposed are those individuals who developed the outcome of interest is unknown. Cross-sectional studies, although they provide information on individuals, are also restricted to being hypothesis-generating because they do not ensure that exposure has preceded disease; an essential criterion to support causal inference (Hill 1965).

Cohort and case-control studies provide the individual level of assessment necessary to support causation while ensuring exposure precedes disease and allowing for adjustment for confounding. Individuals are enrolled into case-control studies based on their disease status (diseased or not diseased) at the time the study is initiated. These individuals are then surveyed to assess their retrospective exposure status. Because of the nature of this study design, case-control studies are best suited for studying multiple exposures, rare diseases, and diseases with long latency periods. That participants are selected according to disease status in a case-control study makes it impossible to calculate a true RR because the study participants are not representative of the entire population so that true incidence of disease cannot be known. Therefore, the formula for RR (Equation 2) cannot be used in case-control studies. An estimate of the RR can be obtained from calculation of the odds ratio (OR) (Hennekens and Buring 1987). The formula for the OR uses the same exposure and disease categories as outlined in Table I-2 and is calculated according to the following equation:

$$RR \cong OR = \frac{a/c}{b/d} = \frac{ad}{bc} = \frac{\text{odds of exposure among cases}}{\text{odds of exposure among controls}} \quad (3)$$

Cohort studies enroll participants who are free of disease at the start of the study based on exposure status (exposed or not exposed). The individuals are then followed into the future (prospective cohort) or tracked from the past to the present (retrospective cohort) to determine if they do or do not develop the outcome of interest. The nature of its design allows for more accurate ascertainment of exposure status using a prospective cohort and this design is most applicable for studying outcomes with short latency periods. Given adequate individual exposure assessment, cohort and case-control studies are capable of testing a hypothesis of causation. Prospective cohort studies are the most desirable for studying if there is a causal relationship between DBPs and adverse reproductive outcomes because the short and defined exposure period for reproductive effects allows for prospective exposure assessment.

## *Review of the Epidemiologic Literature on Disinfection By-products and Adverse Reproductive Outcomes*

Research on a possible relationship between adverse reproductive effects and exposure to DBPs began after a series of studies reported increased tap water consumption was associated with high rates of spontaneous abortions in two California census tracts, independent of water contamination with organic solvents that were the original focus of the studies (Deane et al. 1989; Swan et al. 1989; Deane et al. 1992; Fenster et al. 1992; Windham et al. 1992; Wrensch et al. 1992). The epidemiologic studies that have followed this initial research have specifically concerned DBP exposure and various adverse reproductive outcomes and are, consequently, the studies that are reviewed here. Although these studies could conceivably be classified with respect to study design, reproductive effects observed, or exposure assessment method, they will be reviewed according to exposure assessment because this aspect forms the basis of the rationale for using biomarkers of exposure to improve understanding of the relationship between DBPs and adverse reproductive outcomes.

### *Use of Water Source or Water Treatment Method to Assess Exposure*

Classifying exposure based on the comparison of the type of water source (surface water vs. ground water) or the treatment practice (chlorinated vs. non-chlorinated) is the simplest form of exposure assessment used in the current epidemiologic literature. Three of the recent epidemiologic studies available have used this method of assessing exposure (Aschengrau et al. 1993; Magnus et al. 1999; Yang et al. 2000).

Aschengrau et al. (1993), in a case-control study conducted in Massachusetts, investigated the relationship between drinking water source (ground water vs. surface water) and disinfection method (chlorination vs. chloramination) and the occurrence of neonatal deaths, stillbirths, and congenital anomalies. Exposure evidence for this study was gathered by assigning each case and control to a water source or treatment practice based on records from the Massachusetts Department of Environmental Protection for the community in which women lived at the time of delivery. The authors note that all Massachusetts surface water is treated by either chlorination or chloramination and that

ground water is generally left untreated. Untreated ground water is expected to contain no DBPs as it is often not disinfected with chlorine and even if it is, ground water generally contains low levels of the organic precursors required to form DBPs. Likewise, chloraminated water is expected to contain fewer DBPs than chlorinated water because chloramine is less reactive with organic precursors than is free chlorine. Chloramine is a weaker oxidant than chlorine but has a more stable residual that results in a larger contact time. Therefore, chloramine does not need to be added in as large a quantity as chlorine or as frequently, resulting in fewer DBPs being formed. In contrast, chloramine is a weaker disinfectant, so longer contact times are necessary to achieve disinfection equivalent to free chlorine. This means that those subjects who received surface water or chlorinated water were considered 'exposed' and those who received ground water or chloraminated water were classified as 'non-exposed'. Results from the Aschengrau et al. study show that there was no relationship between water source and congenital anomalies (adjusted OR = 1.0), stillbirths (adjusted OR = 1.0), or neonatal deaths (adjusted OR = 0.90) as all of the RRs were 1.0 or below. When comparing chloraminated vs. chlorinated surface water a fairly high excess of respiratory (OR = 3.2, CI = 1.1 – 9.5) and urinary tract defects (OR = 4.1, CI = 1.2 – 14.1) was found but there was not a significant excess of all congenital anomalies (OR = 1.0), stillbirths (adjusted OR = 2.9, CI = 0.90 – 7.5), or neonatal deaths (OR = 1.0) (Table I-4).

Magnus et al. (1999) conducted a case-control study to investigate exposure to DBPs and the occurrence of birth defects. Specific defects considered were neural tube, major cardiac, respiratory, urinary, and oral cleft defects. Exposure was assessed according to chlorination level and color. Color was used as an indicator for natural organic matter with the hypothesis that an increasing amount of humic matter leads to an increased level of DBPs formed when water is chlorinated. Therefore, an increased number of defects would be expected in areas where color is high and water is chlorinated. Cases and controls were assigned to the exposed and reference groups according to mother's place of residence at the time of delivery. Comparing exposed (high color, chlorination) and reference groups (low color, no chlorination) a positive association (adjusted OR = 1.99, CI = 1.10 – 3.57) was only shown for urinary tract defects (Table I-4).

The most recent study using this surrogate method of assessing exposure was a retrospective cohort study performed by Yang et al. (2000) in Taiwan. The outcomes of interest were term low birth weight (TLBW) (≥37 weeks gestation, <2,500 g) and pre-term delivery (< 37 weeks gestation). Exposure was assessed according to whether the municipality in which a mother lived at the time of delivery did or did not receive chlorinated water. A municipality was considered as a chlorinating municipality (CHM) if greater than 90 percent of the municipal population was served by chlorinated water. Likewise, a municipality was considered non-chlorinating (NCHM) if greater than 95 percent of the municipal population received water from non-chlorinated sources. The CHMs and NCHMs were matched according to similar levels of urbanization to control for confounders resulting from differing levels of urbanization. Comparing CHMs to NCHMs there was no increased risk of term low birth weight (adjusted OR = 0.90, CI = 0.75 – 1.09), however, there was a slightly increased risk of pre-term delivery (adjusted OR = 1.34, CI = 1.15 – 1.56) (Table I-4).

Using water source or water treatment method is a relatively crude method of exposure assessment. The major limitations of using this methodology are that specific DBPs are not measured, volumes of water consumed and beverages other than drinking water are not considered, and corrections for mothers moving residences during pregnancy are not performed. One other significant limitation is there is no assessment of the inter-individual variation in exposure to DBPs because of the different ways and amounts in which individuals use water.

*Use of Routinely Measured DBP Concentrations to Assess Exposure*

An improvement to using water source or treatment method for exposure assessment has been to use specific DBP concentrations routinely measured at water treatment facilities and assigning these concentrations to women based on the facility that served their residence during their pregnancy. Currently, five epidemiologic studies have used this exposure assessment methodology (Kramer et al. 1992; Bove et al. 1995; Gallagher et al. 1998; Dodds et al. 1999; King et al. 2000).

Kramer et al. (1992), in a population-based case-control study conducted in Iowa, investigated the relationship between exposure to individual THMs and low birth weight (LBW) (<2,500 g), prematurity (<37 weeks gestation), and intrauterine growth retardation (IUGR) (<5$^{th}$ percentile of weight for gestational age). The cases and controls for this study were selected from January 1, 1989 to June 30, 1990. However, individual THM levels for chloroform, CDBM, BDCM, and bromoform were determined from a 1987 survey of municipal water quality and these concentrations were assigned to women based on maternal residence at the time of delivery. Thus, the water data did not correspond to the time period in which the adverse reproductive outcomes were assessed. The only significant association that remained after adjusting for potential confounders was an increased risk of IUGR where chloroform concentrations were ≥10 μg/L (OR = 1.8, CI = 1.1 – 2.9) (Table I-4), a very low level of chloroform.

A cross-sectional study, using a similar method of assessing exposure, was carried out by Bove et al. (1995) in New Jersey. The outcomes of interest studied were TLBW (<2,500 g, ≥37 weeks gestation), fetal deaths, small for gestational age (SGA), prematurity (<37 weeks gestation), very low birth weight (VLBW) (<1,500 g), and birth defects that occurred during the period 1985-1998. Individual birth defects investigated were central nervous system (CNS), neural tube, cardiac, and ventricular septal defects. Quarterly total trihalomethane (TTHM) levels were obtained from the Bureau of Safe Drinking water for the period of 1984-1988 and from these data monthly estimates were calculated and assigned to each gestational month. The TTHMs were the mathematical sum of the concentrations of the individual THMs: chloroform, CDBM, BDCM, and bromoform. The average of first trimester exposures was used for birth defect outcomes and fetal deaths. Exposures for all other pregnancy outcomes used TTHM levels averaged over the entire pregnancy. Results for TTHM exposures greater than 100 μg/L showed a significant relationship for term low birth weight (OR = 1.42, CI = 1.22 – 1.65) and SGA (OR = 1.50, CI = 1.36 – 1.65). No associations were determined for prematurity, VLBW, or fetal deaths. It is important to note than a decreased incidence for all defects was shown for TTHM levels greater than 100 μg/L, showing no trend between an increasing level of exposure and an increased level of all birth defects. For exposures to TTHMs greater than 80 μg/L, significant relationships were shown for all defects (OR = 1.57, CI

= 1.23 – 1.99), CNS (OR = 2.59, CI = 1.53 – 4.30), and neural tube defects (OR = 2.96, CI = 1.26 – 6.62) (Table I-4). However, because this was a cross-sectional study it is, at best, limited to being hypothesis-generating because its design cannot assume that exposure preceded disease.

Gallagher et al. (1998) used a retrospective cohort design to study the relationship between TTHMs and LBW (<2,500 g), TLBW (<2,500 g, ≥37 weeks gestation), and pre-term delivery (< 37 weeks gestation) in Colorado. Using a hydraulic characterization procedure, quarterly TTHM data was used to assign exposures to women with respect to time and location of residence in census block groups for the third trimester of pregnancy. Data were evaluated for the period of January 1, 1990 through December 31, 1993. For the highest exposure level (≥61 µg/L TTHM), a weak association was shown for LBW (adjusted OR = 2.1, CI = 1.0 – 4.8) but there was a large increase in TLBW (adjusted OR = 5.9, CI = 2.0 – 17.0) for the same exposure level. No relationship was shown between pre-term delivery and exposure to TTHMs (Table I-4).

In a retrospective cohort study conducted in Nova Scotia, Dodds et al. (1999) examined if a correlation existed between TTHMs and LBW (<2,500 g), VLBW (<1,500 g), pre-term delivery (<37 weeks gestation), SGA, stillbirths, and congenital anomalies. The specific anomalies studied were neural tube, cleft lip and palate, cardiac, and chromosomal abnormalities. From quarterly TTHM data, linear regression was used to estimate monthly TTHM values and the mother's address at the time of delivery was linked to the water quality data for the water facility that served that area. TTHM exposures for the last three months of pregnancy were used for pregnancy outcomes related to fetal growth and time of delivery. The average exposure for the last two months of pregnancy was used for cleft lip and palate and cardiac defects. Average TTHM values for the one month preceding and following conception were used for neural tube defects and for chromosomal abnormalities, the average of the TTHM values for the three months preceding pregnancy was used. TTHM values averaged over the entire pregnancy were used for stillbirth outcomes. No association was found between TTHMs and LBW, VLBW, pre-term delivery or SGA. There was an increased risk of stillbirth for exposure to TTHMs ≥100 µg/L as compared to TTHM levels of 0-49 µg/L (adjusted OR = 1.66, CI

= 1.09 – 2.52) (Table I-4), but there were no significant relationships for exposure to TTHMs and any of the congenital anomalies.

Most recently, King et al. (2000) have used routinely measured data on individual and total THMs to assess exposure. This study used the same outcome data as the Dodds et al. (1999) study to evaluate the risk of exposure to individual THMs and stillbirth. Least-squares regression was used to transform quarterly data for the individual DBPs chloroform, CDBM, BDCM, and bromoform to monthly data. All monthly values over the pregnancy duration were averaged and this value was matched to each subject using the data for the water facility that served her residence at the time of her delivery. The TTHM levels were calculated and assigned to each participant in the same manner. Bromoform and CDBM were not included in their analysis because these compounds occurred at very low concentrations. After adjusting for potential confounders, TTHMs (adjusted RR = 1.66, CI = 1.09 – 2.54), chloroform (adjusted RR = 1.56, CI = 1.04 – 2.34), and BDCM (adjusted RR = 1.98, CI = 1.23 – 3.49) were all associated with increased risk of stillbirth where BDCM showed the highest risk (Table I-4).

The main limitations with assigning exposure to DBPs based on routinely collected monitoring data is that this method does not take into account fluctuating DBP concentrations that occur in an area, mobility during pregnancy, or variation in DBP exposure from the different ways and amounts of water which people use. Beverages other than tap water (bottled water, commercially prepared beverages) were not considered either. Also, only THM exposures were used and exposures to other DBPs were not considered. This assumes that THM concentrations that are measured at treatment facilities are representative surrogates of exposure to all DBPs throughout the distribution system, which is not necessarily true for all water distribution systems (Rizak et al. 2000). One benefit of these studies is that attempts were made to ascertain exposures for the etiologically relevant periods of fetal development for the observed adverse reproductive effects.

The needs for regulatory monitoring of DBP concentrations and for classifying exposures for epidemiologic investigation are different. Water treatment facilities are required by

regulation to routinely monitor DBPs to ensure that exposures of all consumers are kept less than the regulated or guideline levels. In comparison, exposure assessment for epidemiologic investigation requires knowing actual individual exposure. Therefore, the regulatory monitoring performed by water treatment facilities are not necessarily valuable for assessing individual exposures.

*Use of Routinely Measured DBP Concentrations and Water-use Questionnaires to Assess Exposure*

Improvements to using water quality data have been achieved by assessing water consumption and usage with questionnaires and combining these exposure methods with routinely collected water quality data to get a more accurate estimate of exposure. Three studies, all conducted in the United States, have used this method of exposure assessment (Savitz et al. 1995; Swan et al. 1998; Waller et al. 1998).

The first study to use this method of exposure assessment was a case-control study conducted by Savitz et al. (1995) in North Carolina. Adverse reproductive outcomes considered were miscarriage, pre-term delivery (<37 weeks gestation), and LBW (<2,500 g). Participants were asked retrospectively, via interview, their primary drinking water source at home (water company, well water, or bottled water) and the approximate number of glasses of water drank per day when pregnant. A subject's address at the time of delivery was used to assign her to a public water supplier and the dates of her pregnancy were used to assign quarterly average TTHM values from the correct supplier. The TTHM dose was calculated as the number of glasses of water consumed per day multiplied by the TTHM concentration determined for her residence. From the results, water source was not related to any outcome, increasing amounts of ingested water were associated with decreased risks for all outcomes, and TTHM dose was not related to any adverse outcome except increased risk of miscarriage in the highest sextile of TTHM concentration (adjusted OR = 1.7, CI = 1.1 – 2.7) which was not a part of the overall dose-response gradient (Table I-4).

Waller et al. (1998) conducted a prospective cohort study in three regions of California to determine if there was an association between spontaneous abortion (pregnancy loss at ≤

20 weeks completed gestation) and exposure to DBPs. Using a subject's address at the time of eight weeks gestation, the drinking water facility that served her residence was determined. The average of all individual THM (chloroform, BDCM, CDBM, and bromoform) and TTHM values taken by a subject's water utility for their distribution system during her first trimester was used to assign drinking water concentrations to each subject. From interviews, the subject's daily cold tap water intake was estimated at eight weeks gestation. The results showed that women who drank greater than or equal to five glasses of water per day containing greater than or equal to 75 μg/L TTHM had an increased risk of spontaneous abortion as compared to women who drank less than five glasses of cold water per day containing less than 75 μg/L TTHM (adjusted OR = 1.8, CI = 1.1 – 3.0). Of the four individual THMs, only high BDCM (≥5 glasses per day of cold tap water containing ≥18 μg/L BDCM) was associated with an increased risk of spontaneous abortion (adjusted OR = 2.0, CI = 1.2 – 3.5) (Table I-4).

Using the same Californian locations, Swan et al. (1998) conducted a prospective cohort study to examine the relationship between water consumption and spontaneous abortion (pregnancy loss at ≤ 20 weeks completed gestation). Water consumption practices during pregnancy were assessed with an interview and included questions on the amount of cold tap water (or drinks made from tap water) consumed at home, the amount of hot water (or drinks made from heated tap water) consumed at home, and the amount of bottled water consumed. How tap water was consumed, use of water filter/purifier, water company and bottled water brand, and the number of weekly showers and length were also assessed. Positive associations between increased tap water use and spontaneous abortion were shown for one of the three regions. For this region, comparing high cold tap water consumption (≥6 glasses of water per day) to no tap water consumption, risk of spontaneous abortion was increased (adjusted OR = 2.17, CI = 1.22 – 3.87). Likewise, women with high tap water and no bottled water consumption were at increased risk of having a spontaneous abortion as compared to those women with high bottled water and no cold tap water consumption (adjusted OR = 4.58, CI = 1.98 – 10.64). In contrast, those women who had high bottled water and no tap water consumption had a decreased risk of having a spontaneous abortion compared to those women with high cold tap water and no bottled water consumption (adjusted OR = 0.22, CI = 0.09 – 0.51) (Table I-4).

Although combining water consumption information with routinely collected DBP concentrations is an improved way of assessing exposure over the other exposure assessment methods used, it relies on reporting by subjects to estimate individual exposure. Likewise, because the concentration of DBPs is not measured at the consumer's tap there is additional uncertainty about individual exposures. Variables still unaccounted for are the variation in concentrations of DBFs at different points in the distribution system, at different times of the year, and as a result of differences in water consumption and use. Furthermore, humans are not only exposed to DBPs by drinking chlorinated tap water but also by inhalation and dermal absorption (Weisel et al. 1999); future exposure assessment methods must encompass this reality.

The available reproductive epidemiology studies have relied primarily on TTHM concentrations to assess exposure to DBPs due to the wide availability of this data from routine monitoring. Because the specific by-product(s) responsible for adverse reproductive effects is unknown it is questionable that TTHM concentrations are an appropriate surrogate for exposure to all DBPs. Toxicologic data suggests DCAA, TCAA, and certain haloacetonitriles may be responsible for adverse reproductive effects (Smith et al. 1987; Smith et al. 1988; Smith et al. 1989; EPA/ILSI 1993). Furthermore, available evidence suggests TTHM concentrations may or may not be useful predictors of other DBP concentrations, depending on the individual characteristics of the treatment plant and distribution system (Rizak et al. 2000). Future epidemiologic studies on exposure to DBPs other than the THMs and adverse reproductive outcomes are required.

Together, the current epidemiologic studies are inadequate to infer causality to a reliable degree. However, they are suggestive of a weak association between DBPs and adverse reproductive outcomes. The potential for misclassification bias requires more accurate exposure assessment in epidemiologic studies because if the RRs are more certain, any causal relationship, even if RR is small, is important to public health because of the ubiquitous exposure to some level of DBPs in drinking water. Therefore, given the need for individual exposure assessment and the complexities of exposure to DBPs, the potential for exposure biomarkers to improve exposure assessment in epidemiologic studies requires validation.

Table I-4. Summary of epidemiologic studies based on exposure assessment.

***Exposure type: water source, treatment method***

| Author (year) | Study design, location | Outcomes studied | Exposure Assessment | Main Results[1] |
|---|---|---|---|---|
| Aschengrau et al. (1993) | Case-control, Massachusetts | Congenital anomalies<br>Stillbirths<br>Neonatal deaths | Matched women to water source (surface vs. ground) and water treatment (chlorination or chloramination) at time of delivery. | Ground vs. surface[2]:<br>All anomalies:AOR=1.0<br>Stillbirths:AOR=1.0<br>Neonatal death:AOR=0.90<br><br>Chlorination vs. chloramination:<br>All anomalies:OR=1.0[2,3]<br>Respiratory:OR=3.2[3], CI=1.1-9.5<br>Urinary:OR=4.1[3],CI=1.2-14.1<br>Stillbirth:AOR=2.6, CI=0.9-7.5<br>Neonatal death:OR=1.1[2,3] |
| Magnus et al. (1999) | Retrospective cohort, Norway | Birth defects<br>Neural tube<br>Major cardiac<br>Respiratory<br>Urinary<br>Oral cleft | Matched women to chlorination (yes vs. no) and color (high vs. low) at time of delivery. | Chlorine, high color vs. no chlorine, low color:<br>All defects:AOR=1.14, CI=0.99-1.31)<br>Urinary:AOR=1.99, CI=1.10-3.57 |
| Yang et al. (2000) | Case-control, Taiwan | TLBW<br>Pre-term delivery | Based on whether municipality in which mother lived did or did not receive chlorinated water at time of delivery. | Chlorination vs. no chlorination:<br>TLBW:AOR=0.90, CI=0.75-1.09<br>Pre-term delivery: AOR =1.34, CI=1.15-1.56 |

1. All confidence intervals (CI) are 95% unless otherwise specified.
2. Confidence intervals not available.
3. Adjusted OR not available.

Table I-4. Summary of epidemiologic studies based on exposure assessment continued.

***Exposure type: routinely measured DBP levels***

| Author (year) | Study design, location | Outcomes studied | Exposure Assessment | Main Results[1] |
|---|---|---|---|---|
| Kramer et al. (1992) | Population-based case-control, Iowa | LBW<br>Prematurity<br>IUGR | Matched women to levels of individual THMs from municipal water survey based on maternal residence at time of birth. | Chloroform ≥10 µg/L:<br>IUGR: OR[3]=1.8, CI=1.1-2.9 |
| Bove et al. (1995) | Cross-sectional, New Jersey | TLBW<br>SGA<br>Prematurity<br>VLBW<br>Fetal deaths<br>Birth defects<br>CNS<br>Neural tube<br>Oral cleft<br>Cardiac<br>Ventricular septal | Monthly estimates of TTHMs were determined from quarterly TTHMs and assigned to each gestational month. The average of first trimester exposures was used for fetal deaths and birth defects. Other outcomes used exposures averaged over entire pregnancy. | TTHM >100 µg/L:<br>TLBW:OR[3]=1.42, CI[4]=1.22-1.65<br>SGA:OR[3]=1.50, CI[4]=1.36-1.65<br><br>TTHM >80 µg/L:<br>All defects:OR[3]=1.57, CI[5]=1.23-1.99<br>CNS:OR[3]=2.59, CI[5]=1.53-4.30<br>Neural tube:OR[3]=2.96, CI[5]=1.26-6.62 |
| Gallagher et al. (1998) | Retrospective cohort, Colorado | LBW<br>TLBW<br>Pre-term delivery | Using a hydraulic characterization procedure, quarterly TTHM data was for the third trimester was assigned to each woman based on the census block in which she lived during that time. | TTHM ≥61 µg/L:<br>LBW:AOR=2.1, CI=1.0-4.8<br>TLBW:AOR=5.9, CI=2.0-17.0<br>Pre-term delivery: AOR=1.0, CI=0.3-2.8 |

4. Confidence intervals are 50%
5. Confidence intervals are 90%

Table I-4. Summary of epidemiologic studies based on exposure assessment continued.

***Exposure type: routinely measured DBP levels continued***

| Author (year) | Study design, location | Outcomes studied | Exposure Assessment | Main Results[1] |
|---|---|---|---|---|
| Dodds et al. (1999) | Retrospective cohort, Nova Scotia | LBW<br>VLBW<br>Pre-term delivery<br>SGA<br>Stillbirth<br>Congenital anomalies<br>Neural tube<br>Cleft lip and palate<br>Cardiac<br>Chromosomal | Monthly TTHM levels were estimated from quarterly TTHM levels using linear regression and assigned to a subject's based on address at time of delivery. Different exposure intervals were considered for different outcomes based on the exposure interval that was considered relevant. | TTHM ≥100 µg/L vs TTHM 0-49 µg/L:<br>Stillbirth: AOR=1.66, CI=1.09-2.52 |
| King et al. (2000) | Retrospective cohort, Nova Scotia | Stillbirth | Least-squares regression was used to estimate monthly individual and total THM levels from quarterly data measured by drinking water facilities. The average of the monthly estimates over the pregnancy duration was the exposure estimate assigned to each participant based on the facility that served her residence at the time of delivery. | TTHM ≥100 µg/L:<br>Stillbirth:ARR=1.66, CI=1.09-2.54<br><br>Chloroform ≥100 µg/L:<br>Stillbirth:ARR=1.56, CI=1.04-2.34<br><br>BDCM ≥20 µg/L:<br>Stillbirth:ARR=1.98, CI=1.23-3.49 |

Table I-4. Summary of epidemiologic studies based on exposure assessment continued

***Exposure type: routinely measured DBP levels and water use questionnaires***

| Author (year) | Study design, location | Outcomes studied | Exposure Assessment | Main Results[1] |
|---|---|---|---|---|
| Savitz et al. (1995) | Case-control, North Carolina | Miscarriage<br>Pre-term delivery<br>LBW | Average quarterly TTHM values were matched to women based on residence at time of delivery. Water use was acquired by interviews. TTHM dose equals glasses of water per day x THM concentration. | Highest sextile of THM concentration:<br>Miscarriage:AOR=1.7, CI=1.1-2.7 |
| Waller et al. (1998) | Prospective cohort, California | Spontaneous abortion | Using subject's address during first trimester, quarterly data for total and individual THMs was assigned to each woman. This was combined with water consumption practices at 8 weeks gestation, as assessed by an interview during the first trimester. | ≥5 glasses, ≥75 µg/L vs. <5 glasses, <75 µg/L:<br>AOR=1.8, CI=1.1-3.0<br><br>BDCM (≥5 glasses, ≥18 µg/L):<br>AOR=2.0, CI=1.2-3.5 |
| Swan et al. (1998) | Prospective cohort, California | Spontaneous abortion | Water consumption practices at eight weeks gestation were assessed by an interview conducted during the subject's first trimester. | High cold tap water (≥6 glasses) vs. none:<br>AOR=2.17, CI=1.22-3.87<br>High cold tap, no bottled vs. high bottled, no tap<br>AOR=4.58,CI=1.98-10.64<br>High bottled, no tap vs. high tap, no bottled:<br>AOR=0.22, CI=0.09-0.51 |

*Biomarkers of Exposure*

On the bioavailability continuum from external exposure through external dose to internal dose, internal dose is the most individualized measure of exposure. Therefore, measures of internal dose are most desirable for accurately monitoring individual exposure to DBPs from drinking water. Biomarkers of exposure are parent compounds or their metabolites that show a quantifiable relationship to the amount of compound to which an individual was exposed. Using the above bioavailability terminology, biomarkers of exposure represent measures of internal dose. Thus, if DBPs or their respective metabolites can be measured in human biological media (blood, urine, breath, sweat, saliva) and these measurements correlate to levels of DBPs in drinking water there is potential for exposure biomarkers to provide individual exposure assessment in studies of the relationship between DBPs and adverse reproductive effects.

In order for a compound to be a valid and feasible exposure biomarker for DBPs it should meet most of the following specifications (Froese et al. Submitted):

1. Persistent enough to cover successive exposure intervals.
2. Easily detected in exposed populations.
3. Present at low levels in the unexposed population.
4. Has a high positive predictive value (changes in biomarker levels reflect changes in exposure levels
5. Specific to the exposure source being studied.
6. Biologically relevant to the exposure being studied.
7. Non-invasive and easy to collect.
8. Economical and easy sampling.
9. Stable in storage.

Only recently have attempts been made to assess and validate individual biomarkers of exposure to DBPs. Because the THMs and the HAAs are the most prevalent DBPs in nearly all chlorinated water, these two classes of DBPs have received the most attention as possible biomarkers of exposure. Of these two classes of DBPs, TCAA, one of the HAAs, has shown the most promise as a valid biomarker of exposure to DBPs when measured in urine (Kim et al. 1999; Weisel et al. 1999; Froese et al. Submitted). Specific discussion of these studies is reserved for Chapters II and III. Working from the results provided by these earlier studies, the potential for TCAA to serve as a valid and feasible

biomarker for exposure to DBPs, according to the criteria outlined above, was further investigated for this thesis.

***Analytical Theory***

The routine analysis of TCAA in water has historically been performed using Method 552.2 developed by the United States Environmental Protection Agency (USEPA). This procedure involves concentration, liquid-liquid extraction of the acids from water into an organic phase, and analysis using gas chromatography (GC) (USEPA 1995). Derivitization, the conversion of nonvolatile TCAA to a volatile methyl ester, is a necessary intermediate step between extraction and analysis to enable TCAA analysis by gas chromatography.

The concentration, extraction, derivitization, and separation techniques required by this method are not only labor intensive and time consuming, but also involve using large quantities of solvents that can be toxic and possibly carcinogenic. Furthermore, losses occur due to the concentration step. For these reasons, alternative methods for TCAA analysis in water that use fewer solvents have recently been explored. Of the recently explored methods, solid phase microextraction (SPME) combined with GC is suitable for TCAA analysis in water and biological samples (Sarrion et al. 1999; Wu et al. 2001). The SPME technique involves essentially two steps. In the first step a coated fused-silica fiber is exposed to a prepared (derivitized) sample or its headspace to draw analytes from a gaseous or liquid phase onto the coating. The fiber containing the target analytes is then moved into an analytical instrument for desorption, separation, and quantification (Pawliszyn 1997). Separation is well achieved for the mixture of haloacetic acids encountered in water and urine samples using GC. In GC, the sample is vaporized at a high temperature, injected onto a chromatographic column, and carried through the column by the flow of an inert gaseous mobile phase. The mobile phase does not interact with molecules of the analyte; its only function is to move the analyte through the column. In the column, the sample partitions between the gaseous mobile phase and the liquid phase immobilized on the surface of an inert solid, thereby isolating the target compounds in the sample. The optimum column temperature depends upon the boiling

point of the sample and the degree of the separation required. Roughly, a temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time. For samples with a broad range of boiling points, temperature programming is used, whereby the column temperature is increased either continuously or in steps as the separation proceeds (Skoog and Leary 1992).

Gas chromatography requires a detector for quantification of the analytes of interest. Electron capture detection (ECD) is highly sensitive to organic samples that contain electronegative functional groups such as the halogens. In this type of detector a beta emitter (usually $^{63}Ni$) causes ionization of a carrier gas, producing a constant stream of electrons. In the absence of organic species, this ionization process produces a standing current between a pair of electrodes. The current decreases in the presence of electronegative species or functional groups that capture electrons causing a drop in the potential measured. The chromatogram appears as a mirror image of the potential drops (Skoog and Leary 1992). Because of its applicability to TCAA analysis, headspace SPME combined with GC-ECD was used for the analysis of water and urine samples in this thesis.

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# II Determination of Elimination Half-life for TCAA in a Controlled Human Exposure Study

## *Introduction*

Disinfection of drinking water with chlorine is a primary public health intervention, which results in a reduced incidence of waterborne disease wherever it is reliably carried out. In the developed world, where drinking water disinfection has been widely practiced since the beginning of the twentieth century, deaths from acute infectious diseases transmitted in drinking water have been virtually eliminated except in cases of disinfection failure. This has made drinking water disinfection one of the most important public health practices for the control of infectious disease.

Although chlorine has the benefit of inactivating pathogens in water intended for human use, it also forms by-products by interacting with organic compounds in source water (Bellar et al. 1974; Rook 1974). The first disinfection by-products (DBPs) identified were trihalomethanes (THMs); a class of chlorinated organic compounds that includes chloroform, chlorodibromomethane (CDBM), bromodichloromethane (BDCM), and bromoform. Many more DBPs have since been identified including classes such as haloacetic acids (HAAs), haloacetonitriles, haloketones, aldehydes, halopicrins, and cyanogen halides and individual compounds such as chloral hydrate, 2,4,6-trichlorophenol, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) (Krasner et al. 1989). These DBPs are ubiquitous in most chlorinated water supplies (Richardson 1998) and available toxicologic evidence suggests some DBPs may be carcinogens, mutagens, and/or teratogens (Bull 1985; EPA/ILSI 1993; Austin et al. 1996; Parrish et al. 1996; James et al. 1997). As a consequence, the question of whether DBPs pose a human health risk has received considerable attention in recent years.

Although initially focused on cancer, more contemporary research on the relationship between DBPs and health has focussed on adverse reproductive outcomes. Toxicological evidence from animal studies shows the following associations between DBPs and adverse reproductive outcomes: chloroform with cleft palate, reduced fetal body weight,

and increased fetal deaths (Registry 1993), bromodichloromethane with reduced fetal body weight (EPA/ILSI 1993), haloacetic acids with cardiac defects and reduced fetal body weight (EPA/ILSI 1993), and acetonitriles with low birth weight, increased fetal death, soft tissue defects, and skeletal malformations (Smith et al. 1988; Smith et al. 1989). Observed in isolation, the toxicologic findings appear suggestive of a relationship between exposure to DBPs and adverse reproductive outcomes. However, data from toxicologic studies is produced by administering individual DBPs at doses that are several orders of magnitude higher than those mixtures of by-products that occur in drinking water. This information, although necessary to determine mechanisms of toxic action, is insufficient to determine if there is a causal relationship between exposure to DBPs and adverse reproductive outcomes in humans. Therefore, epidemiologic studies with humans exposed to DBPs at drinking water levels are needed to support the toxicologic data.

Several epidemiologic studies, in many different settings, have recently linked chlorination by-products to adverse reproductive outcomes including small for gestational age, stillbirth, spontaneous abortion, low birth weight, and congenital anomalies (Bove et al. 1995; Savitz et al. 1995; Gallagher et al. 1998; Swan et al. 1998; Waller et al. 1998; Dodds et al. 1999; King et al. 2000; Yang et al. 2000). To test a causal hypothesis, the epidemiologic method must obtain individual measures of exposure and outcome. The ability of the above studies to demonstrate a causal relationship between exposure to DBPs and adverse reproductive outcomes is hindered by inadequate individual exposure assessment. The exposure evidence for the above studies has been gathered through a combination of water source, chlorination practice, municipal water quality data, and water consumption questionnaires. None of these studies has used achieved true individual monitoring for DBP exposure assessment.

Failing to accurately assess individual exposure has the potential to cause misclassification of exposure. Although initially thought to decrease relative risks towards the null (Birkett 1992), more recent evidence suggests exposure misclassification can have the effect of inflating or deflating the results of epidemiologic studies away from or towards the null (Reif et al. 2000). This means that, if exposure was accurately

assessed, the true relative risk of adverse reproductive outcomes could be higher or lower than the relative risks reported in the current epidemiologic studies if there truly is a causal relationship between exposure to DBPs and the occurrence of adverse reproductive effects. Therefore, there is a need for improved capability of epidemiologic methods to assess individual exposure to by-products of disinfection to determine the true risks that disinfection by-products pose to reproductive health.

Exposure assessment for DBPs is complicated by the many different ways and amounts in which individuals use water. Typical uses of water expose humans to DBPs through three routes: inhalation, ingestion, and dermal absorption. The respective physiochemical properties of individual DBPs further determine the extent to which a certain exposure route contributes to the overall exposure to DBPs. Any exposure assessment method must, therefore, account for all possible overall exposures to DBPs.

A promising approach to improving individual exposure assessment in epidemiologic studies is to validate an exposure biomarker for DBPs. Biomarkers of exposure are parent compounds or their metabolites that show a quantifiable relationship to the total amount of a compound to which an individual is exposed. If DBPs or their metabolites can be measured in biomonitoring media (blood, breath, urine, saliva, or sweat) and these levels correlate to measures of DBPs in drinking water, there is potential for exposure biomarkers to provide valid and feasible individual exposure assessments in epidemiologic studies.

For a biomarker compound to be a valid surrogate of exposure it must be persistent enough in the human body to integrate successive exposure intervals. The duration for which a compound stays in the body is indicated by its elimination half-life. If a compound's elimination half-life is longer than successive DBP exposure intervals, it fulfills this requirement for use as an exposure biomarker. Furthermore, using biomarkers in epidemiologic studies cannot be realized unless they can be feasibly implemented into the study design. This involves the biomarker being relatively non-invasive to collect and that it is technically and economically practical to measure.

Of the many DBPs that are present in chlorinated waters, the THMs and HAAs are the most prevalent by-products (mass basis) and, therefore, have recently been investigated as potential biomarkers of exposure for DBPs. Weisel et al. (1999) found that when measurable levels of THMs were obtained in post-shower breath samples these breath levels correlated to THM levels in water. The majority of the breath samples, however, were non-detectable for THMs in exhaled breath because THMs are rapidly metabolized in the body following inhalation or dermal absorption (Weisel et al. 1992). Trihalomethanes are not persistent enough in the body to provide any information on exposure to DBPs via ingestion because of first-pass metabolism of ingested THMs in the liver (Weisel and Jo 1996). Because THM levels measured in breath are not persistent enough for sufficient time to integrate all exposure measurements over more than minutes to hours they are not viable exposure biomarkers for DBPs.

In a cross-sectional study of 49 women in New Jersey, trichloroacetic acid (TCAA), one of the haloacetic acids, was found to be a promising exposure biomarker when measured in first morning urine (FMU) (Kim et al. 1999; Weisel et al. 1999). Comparing high and low ingestion exposures, higher excretion levels of TCAA were shown at higher exposure levels as compared to excretion levels at low ingestion exposures. Using existing pharmacokinetic literature (Breimer et al. 1974; Humbert et al. 1994), it was suggested that TCAA may have a sufficiently long half-life in the human body to allow internal TCAA concentrations to approach steady state, permitting urinary TCAA excretion to represent TCAA exposure over several days. The available pharmacokinetic data was not produced from the ingestion of TCAA at drinking water concentrations; it was generated from administration at high concentrations of chloral hydrate (CH), which produces TCAA as one metabolite.

To test whether TCAA from drinking water is maintained long enough in the human body to support its use as an exposure biomarker, Froese et al. (Submitted) conducted a longitudinal pilot study to measure the elimination half-life of TCAA in urine. A cohort of 10 participants from a water research laboratory in Adelaide, Australia consumed normal tap water followed by an intervention period where they consumed DBP-free bottled water. First morning urine samples were provided by all volunteers for specific

days of the tap water and bottled water exposures. Volunteers were not restricted from ingesting other beverages made with the tap or bottled waters and they were not asked to abstain from drinking commercially prepared beverages. Due to the low ingestion exposures experienced by most of the participants, elimination kinetics information was only available for three subjects. The half-life values determined were 2.3, 2.9, and 3.7 d with coefficients of determination ($R^2$) of 0.74, 0.70, and 0.45, respectively. The substantial variability in TCAA ingestion and the limited number of data points from which these values were calculated suggested the need for a more closely controlled exposure biomarker study to provide greater understanding of human TCAA elimination kinetics.

This longitudinal pilot exposure/intervention study was designed to measure elimination half-life of TCAA in urine when beverage consumption is limited to water of known TCAA concentration and ingestion volume is managed. Specific goals of the study were to have a sufficient number of data points with which to accurately measure elimination half-life and to control for variations in TCAA exposure from beverages other than water and fluctuating TCAA ingestion volumes. The study was also designed to investigate if TCAA in FMU samples is representative of total daily TCAA excretion and to determine ways in which the practicality of using biomarkers in future epidemiologic studies can be improved.

## *Methods*

### *Volunteer Recruitment*

Volunteers from the University of Alberta in Edmonton, Alberta, Canada were sought for participation in the study. Five volunteers in total, three males and two females, were recruited. The average age of all participants was 35 years (range = 27 – 52 years), that of the male participants was 38 years (range = 27 – 52 years), and that of the female participants was 29 years (range = 28 – 29 years). All of the volunteers included individuals who were either students or staff from our research group, therefore, they had a vested interest in the research and were highly compliant participants.

*Water Source*

Results from the earlier pilot study conducted by Froese et al. (Submitted) in Adelaide, Australia illustrated the importance of having sufficiently high TCAA ingestion exposures to estimate elimination half-life. Drinking water in Edmonton contains very low levels of TCAA (Epcor 2000), therefore, water was imported from Winnipeg, Manitoba, Canada for use in Edmonton because the higher levels of TCAA in Winnipeg water (Rizak et al. 2000) would yield better TCAA excretion responses. Another benefit of Edmonton water having considerably lower levels of TCAA than the Winnipeg water is that inhalation and dermal TCAA exposures from routine household activities using Edmonton water would be less likely to influence the ingestion results.

The difference in TCAA levels between Winnipeg and Edmonton water is a function of the respective water sources and treatment processes in the two cities. Water in Edmonton is drawn from the North Saskatchewan River within the city limits and is treated once with chlorine at the treatment plants. This chlorine is then converted to chloramine before it is sent into the distribution system. Chloramine is a weaker oxidant than chlorine but has a more stable residual time. Because using chloramines avoids having free chlorine in the water, less TCAA is formed (Singer 1993).

Drinking water in Winnipeg originates from Shoal Lake, located on the border of Manitoba and Ontario. Directly after the water is drawn from the lake it is treated with chlorine and then travels through a 160 kilometer aqueduct to a large open reservoir outside of Winnipeg. After sitting in this large reservoir the water is chlorinated again before it is sent to regional reservoirs within the city. From the regional reservoirs the water is chlorinated once more and then travels into the distribution system (Rizak et al. 2000). Because there is a substantial dose of free chlorine added to the water and this chlorine has a long contact time, greater concentrations of DBPs are formed in Winnipeg water than in Edmonton water.

*Study Design*

This study was designed to measure the elimination half-life of TCAA. Therefore, participants consumed normal tap water from Winnipeg for two weeks and then switched to TCAA-free bottled water for two weeks. The TCAA-free bottled water was obtained from Sierra Spring Water Company in Edmonton. One participant remained on bottled water for three weeks for the bottled water intervention. First morning urine (FMU) samples were collected from each volunteer for every day of the study. In addition to collecting all FMU samples, one participant collected all urine samples throughout the day and recorded all urination times. These data were used to calculate the contribution of FMU to total daily TCAA excretion. A summary of the study design is provided in Table II-1.

Table II-1. Study design for TCAA exposure and intervention study.

| Week | Water Source | Sample Days | FMU Samples | All-day Samples |
|---|---|---|---|---|
| 1 | Winnipeg water | 7 | 5 participants | 1 participant |
| 2 | Winnipeg water | 7 | 5 participants | 1 participant |
| 3 | Bottled water | 7 | 5 participants | 1 participant |
| 4 | Bottled water | 7 | 5 participants | 1 participant |
| 5 | Bottled water | 7 | 1 participant | 1 participant |

For the purpose of eliminating TCAA exposures from beverages other than water, participants were asked to limit their beverage consumption to only the water provided. One exception to this request was that volunteers were allowed to drink milk, as milk is not expected to contain DBPs and is an important calcium source in daily diets. The earlier pilot study showed variations in water ingestion volume greatly affected TCAA exposures (Froese et al. Submitted). Thus, to manage ingestion volume, as would be reflected in FMU samples, all volunteers were requested to ingest at least 1000-mL of water after 17:00 hr each day. Ingestion volumes were directly measured with a dedicated measuring cup provided to each volunteer.

The bulk water was shipped and stored, unrefrigerated, in 20-L polycarbonate containers (Nalgene). Glass containers and refrigeration likely offer greater stability for HAAs in unpreserved water. However, polycarbonate was chosen for ease of shipment (as

compared to glass) and to limit the extent to which the taste of the water was altered versus other plastics such as polyethylene. Additionally, many bottled water companies use polycarbonate containers for shipping and storing their products. Participants were also supplied with two plastic containers (Rubbermaid™) to store and transport their drinking water. The water used during the study was not preserved with ammonium chloride because the amount of preservative required for quenching free chlorine (100 mg/L) (U.S. EPA 1995) highly affects the taste and chemistry of drinking water, making it unfeasible to give to human participants. Further, the $LD_{50}$ level for ammonium chloride (30 mg/kg) (Merck 1996), which is the amount required to kill 50% of the rats to which it is given, indicates that this compound should not be used for human consumption at the levels specified in the U.S. EPA method for HAA analysis.

Before sample collection began, urine collection bottles and daily diaries were sent to each volunteer with instructions on how to collect a urine sample and how to fill out the daily diary. The participant recorded the collection times of the urine samples, previous urination times, the time and amount of all fluid consumption, the time and amount of any medications taken, and the time and duration of other specified water related activities. Required water related activities to document were showering, bathing, bathing others, washing dishes or cars, swimming, and steamroom/sauna and hot-tub use. Information on medication use was collected because some medications contain chloral hydrate and TCAA is a metabolite of chloral hydrate (Breimer et al. 1974; Humbert et al. 1994). Participants were also asked to record any visits to dry-cleaning facilities for the purpose of assessing exposures to trichloroethylene (TCE), another compound of which TCAA is a metabolite (Abbas and Fisher 1997). Winnipeg water consumption and the diaries were started 48 hr prior to the first urine sample collection. Water was provided as needed. The data collection was conducted between 3 July 2000 and 4 August 2000.

*Urine Samples*

Entire urine voids were collected in 1000-mL high-density polyethylene bottles and brought to the laboratory by the volunteers themselves. If the volunteer could not deliver the sample to the laboratory within approximately 4 hours, the sample was stored in a

refrigerator or in a cooler with ice. Once at the laboratory, all samples were stored in a refrigerator (4°C) until extraction. All samples were extracted within 24 hr of collection. Separate analyses confirmed TCAA in urine samples was stable for up to two weeks. The volume of each sample was measured in the laboratory. After the volume was measured, 10-mL of each sample was poured into a polyethylene vial and transported to Dynacare Kasper Medical Laboratory (DKML) in Edmonton for creatinine analysis. On average an adult male will excrete creatinine at a rate of 1.7 μg/d and an adult female will excrete creatinine at a rate of 1.0 μg/d (Jackson 1966), therefore, creatinine can be used for the purpose of correcting for variations in urine volume. DKML analyzed creatinine by a colorimetric method using the reaction of creatinine with sodium picrate and UV absorption at 500 nm (DKML 2000). Because creatine, the metabolic precursor of creatinine, is used as an exercise supplement, all participants were assessed for creatine use prior to the start of the study. No participants reported taking supplemental creatine.

TCAA concentrations in the urine samples were measured according to a modified version of EPA Method 552.2 (USEPA 1995) that employed the use of solid phase microextraction (SPME) (Sarrion et al. 1999). A 40-ml sample of urine was measured with a graduated cylinder and poured into a 50-mL centrifuge tube. Into each sample, a surrogate standard was added. The surrogate standard used was 75-μL of a 25 μg/mL solution of 2,3-dibromopropionic acid. Samples were acidified with 2-mL of concentrated sulfuric acid (Fisher Scientific) and then approximately 12-g of anhydrous sodium sulfate (Fisher Scientific) was added. Extraction was achieved by adding 4-mL of methyl tert-butyl ether (MTBE) (PRA Grade, Aldrich). The samples were shaken for nine minutes on a Vibrax-VXR automatic shaker and then centrifuged for 5 minutes at 2500 rpm (Sorvall Biofuge Primo, Mandel Scientific Company).

Using a glass Pasteur pipette, the entire solvent layer was removed, placed in a 10-mL glass vial, and evaporated at 50°C with nitrogen gas (UHP grade) using a heated evaporator apparatus (Pierce Reacti-Vap 18780; Pierce Reacti-Therm). To the evaporated sample, 53-μL of methanol (HPLC grade, Fisher Scientific) and 93-μL of concentrated sulfuric acid (Fisher Scientific) were added to achieve esterification and the

vials were sealed with crimp-tops. The finished sample was then heated at 50°C for 10 minutes before analysis.

SPME was performed with a 100-μm film thickness polydimethylsiloxane (PDMS) fiber housed in an auto-sampler (Varian 8200). The PDMS fiber was exposed to the headspace for 10 minutes to extract the trichloroacetic acid methyl esters. The subsequent desorption time was two minutes at 300°C. Vial blanks were routinely run to ensure full desorption of samples from the PDMS fiber.

Analyses were performed on a Varian 3800 gas chromatograph (GC) with an electron capture detector (ECD). The GC was equipped with a DB-5 column (5% diphenyl and 95% dimethylpolysiloxane; 30 m x0.25 mm i.d., 0.25 um film thickness; J & W Scientific Co.) and helium was used as the carrier gas at a flow-rate of 1 ml/min. The temperature program was 40°C (held for 10 minutes) to 75°C (held for five minutes) at 5° per minute and up to 150°C (held for one minute) at a rate of 20° per minute. A 95% argon/5% methane mix was used for the detector make-up gas at a rate of 28 mL per minute. Inlet and detector temperatures were maintained at 300°C and 350°C, respectively. The GC was operated in the splitless mode and was calibrated with a mixture of methyl ester HAAs (Supelco) (calibration range 1 to 200 μg/L for TCAA). The linear calibration curve used for the urine samples had an $R^2$ value of 0.9992 (Appendix 1). This method for TCAA analysis in urine allowed for sensitive detection of TCAA (method detection limit [MDL] = 0.61 μg/L) and showed excellent repeatability (relative standard deviation of 5.6% on seven triplicate analyses of urine). The MDL was calculated as three times the standard deviation of seven samples of the lowest urine TCAA concentration (Taylor 1987).

*Water Samples*

Each day the participants were consuming the Winnipeg tap water, samples of the tap water were analyzed for TCAA using the same method as for the urine samples except that the samples were not centrifuged. Water used for the TCAA analysis was stored in a refrigerator (4°C) and was analyzed at the same time as the urine samples. The method

used for analyzing TCAA concentrations in water samples showed sensitive detection of TCAA (MDL = 0.82 µg/L) and good repeatability (relative standard deviation of 8.7% on 3 triplicate analyses of water). The MDL was calculated as three times the standard deviation of seven samples of water with a TCAA concentration of 0.1 µg/L (Taylor 1987).

***Results and Discussion***

*Elimination Half-life Estimation Based on Creatinine Adjusted TCAA*

For the elimination half-life ($t_{1/2}$) estimates, the TCAA concentration measured in FMU samples was divided by the creatinine concentrations in these samples to correct for any fluctuations in urine volume according to Equation (1). This corrected TCAA concentration (µg TCAA / g creatinine) was plotted against time in days (Microsoft Excel 2000), with day zero being the average of all days on Winnipeg water and day one being the first day of the bottled water intervention. The logarithm of the creatinine-normalized TCAA urinary excretion was evaluated for linear fit against time (corresponding to one phase exponential decay).

$$\text{Creatinine - normalized TCAA}\left(\frac{\mu\text{g TCAA}}{\text{g creatinine}}\right) = \text{TCAA concentration}\left(\frac{\mu\text{g}}{\text{L}}\right) \div \text{Creatinine concentration}\left(\frac{\text{mmol}}{\text{L}}\right) \times \text{Mol.wt. creatinine}\left(\frac{\text{mmol}}{0.11312\text{g}}\right) \quad (1)$$

Elimination half-life is defined as the time it takes for the concentration of a compound in the body to decline to half of its original value (Clark and Smith 1986). From a linear plot of log concentration versus time, half-life can be determined from the elimination rate constant ($k_{el}$). Using the logarithm of the creatinine-normalized urinary excretion versus time curve, the elimination constant was used to calculate the elimination half-life according to the equation:

$$t_{1/2} = \frac{0.693}{k_{el}} \quad (2)$$

Half-life values for the five participants, as determined from the FMU creatinine-normalized TCAA excretion versus time curves were 2.1, 2.3, 2.5, 5.0, and 6.3 d with coefficient of determination ($R^2$) values of 0.83 (n=14), 0.94 (n=13), 0.88 (n=14), 0.80 (n=20), and 0.76 (n=14), respectively (Figures II-1 to 5). The elimination kinetics determined for TCAA were confirmed to be first order using pharmacokinetic modeling software (GraphPad Prism Version 3).

Observation of the creatinine adjusted TCAA elimination versus time curve for participant WET-1 (Figure II-1) shows an outlier for day 2 of the bottled water intervention. Three days previous to that study day (July 13) the maximum daily temperature was the highest it had been throughout the study duration. Also, on this day, participant WET-1 recorded engaging in outdoor physical activity during the hottest part of the day. On the following day (July 14) the volunteer compensated for the effects of the previous hot day and physical activity by consuming 2.1 times more water than average while excreting a urine volume 2.5 times less than average (Figure II-6). This combination of high ingestion volume and low excretion volume is a possible explanation for the high concentration of TCAA in the urine sample on day two of the bottled water intervention as TCAA excretion is expected to lag TCAA ingestion.

The creatinine adjusted TCAA elimination versus time curve for participant WET-5 also shows an outlier for day two of the bottled water intervention (Figure II-5). Observation of the daily diary for participant WET-5 does not show an increase in water ingestion volume or a decrease in urine excretion volume in response to high ambient temperature. The diary for WET-5 did show that this participant consumed 300-mL of commercially prepared iced tea two days previous to the day with the high TCAA urine sample. Iced tea made with chlorinated water can contain double the amount of TCAA than chlorinated water alone (Balko et al. 2001). The ingestion of iced tea by participant WET-5 possibly explains the high value for this participant for day two of the bottled water intervention, due to the lag between TCAA ingestion and excretion. If we assume

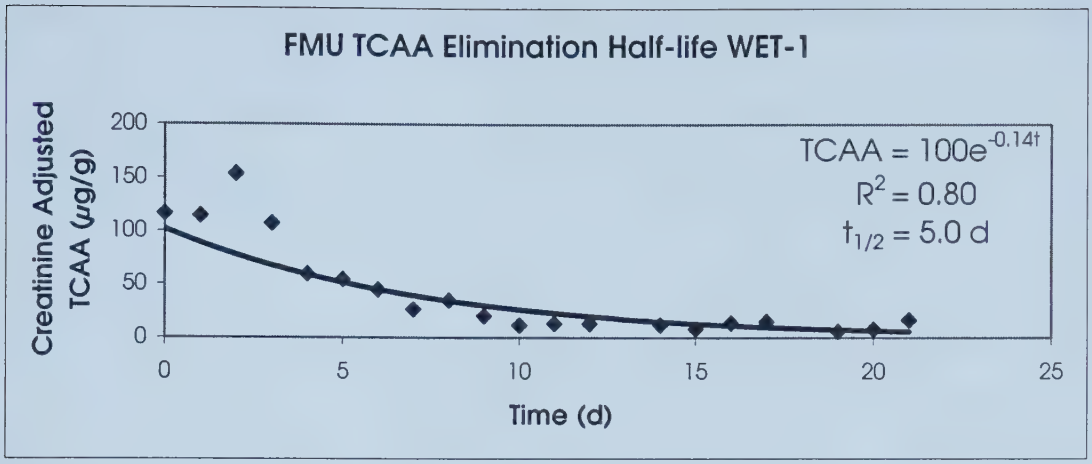


Figure II-1. TCAA elimination half-life estimation for WET-1 using creatinine adjusted TCAA concentration in FMU.

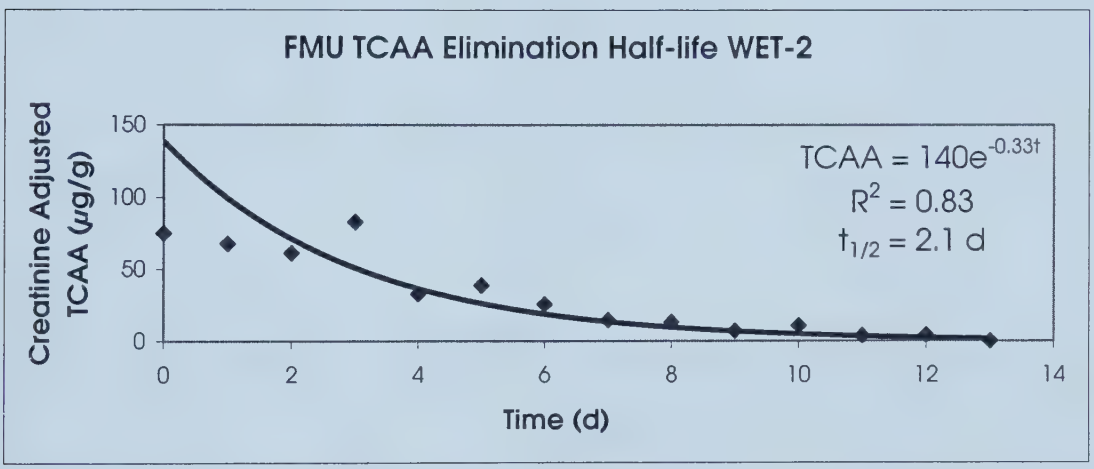


Figure II-2. TCAA elimination half-life estimation for WET-2 using creatinine adjusted TCAA concentration in FMU.

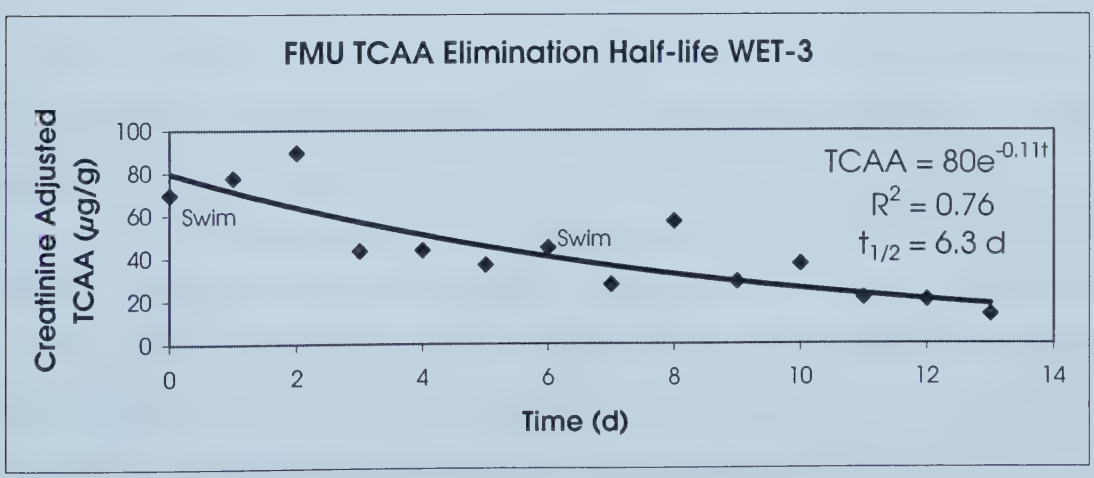


Figure II-3. TCAA elimination half-life estimation for WET-1 using creatinine adjusted TCAA concentration in FMU.

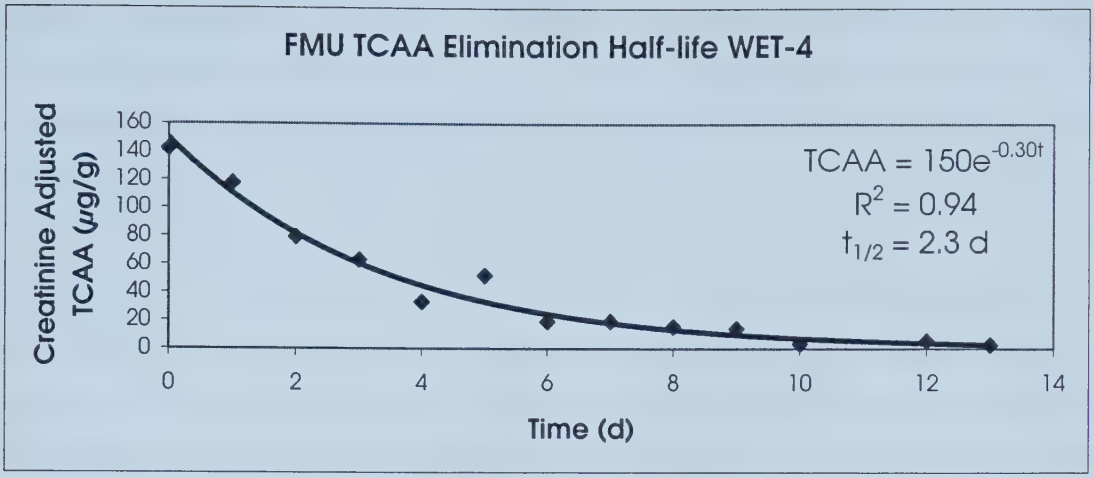


Figure II-4. TCAA elimination half-life estimation for WET-4 using creatinine adjusted TCAA concentration in FMU.

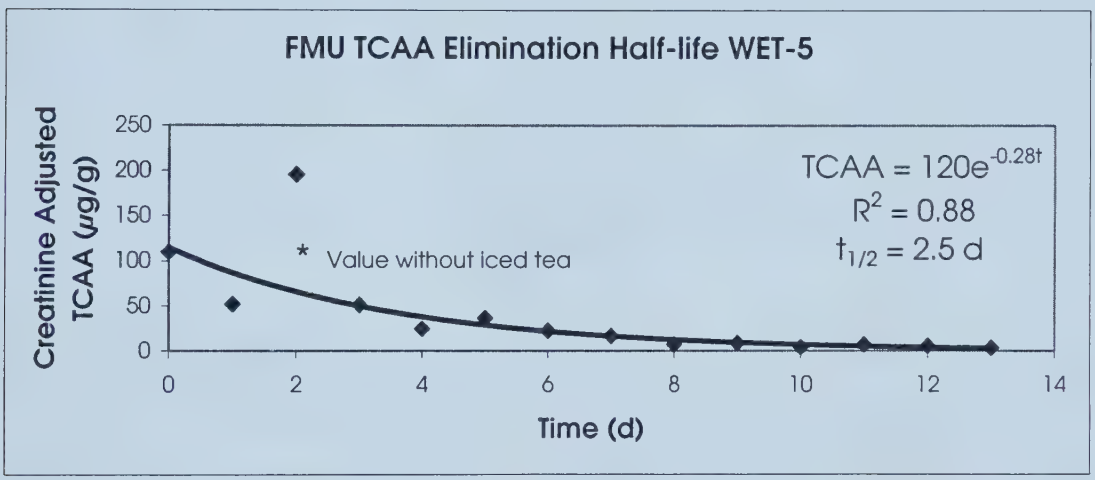


Figure II-5. TCAA elimination half-life estimation for WET-5 using creatinine adjusted TCAA concentration in FMU.

a worst-case scenario, and estimate that the water used to prepare the iced tea contained approximately the same amount of TCAA as the Winnipeg water used (~100 µg/L) an estimated value of 200 µg/L of TCAA would be in the iced tea due to the relative doubling. Knowing that participant WET-5 consumed 300-ml of this iced tea and that this participant has an elimination half-life of approximately two days, an estimated value of 30 µg of TCAA would have been contributed by the consumption of iced tea two days previous [(200 µg/L x 0.3 L) / 2 = 30 µg]. Without the 30 µg of TCAA contributed by the iced tea consumption the creatinine-adjusted TCAA value would be 120 µg/g (Figure II-5). This possible outcome demonstrates the importance of assessing the contribution of all TCAA exposures when studying exposure biomarkers for TCAA.

Similar to participants WET-1 and WET-5, the creatinine adjusted TCAA elimination versus time curve for participant WET-3 showed a curiously high value on day two of the bottled water intervention (Figure II-3). As for participant WET-5, an increase in water volume consumed or a decrease in urine volume excreted does not explain the high value. Analysis of the daily diaries for volunteer WET-3 shows that two days previous to the second day of the bottled water intervention this participant recorded swimming. TCAA concentrations in swimming pools are substantially higher than drinking water concentrations because of the large amount of chlorine required for adequate disinfection and the introduction of additional organic precursors by human activities in the water. Although the contribution of dermal absorption to the dose of TCAA from routine household uses is minor (<1 percent), the high concentration of TCAA in swimming pools combined with the possibility of water ingestion during swimming can increase weekly TCAA exposures by 10 to 20 percent (Kim and Weisel 1998). The TCAA

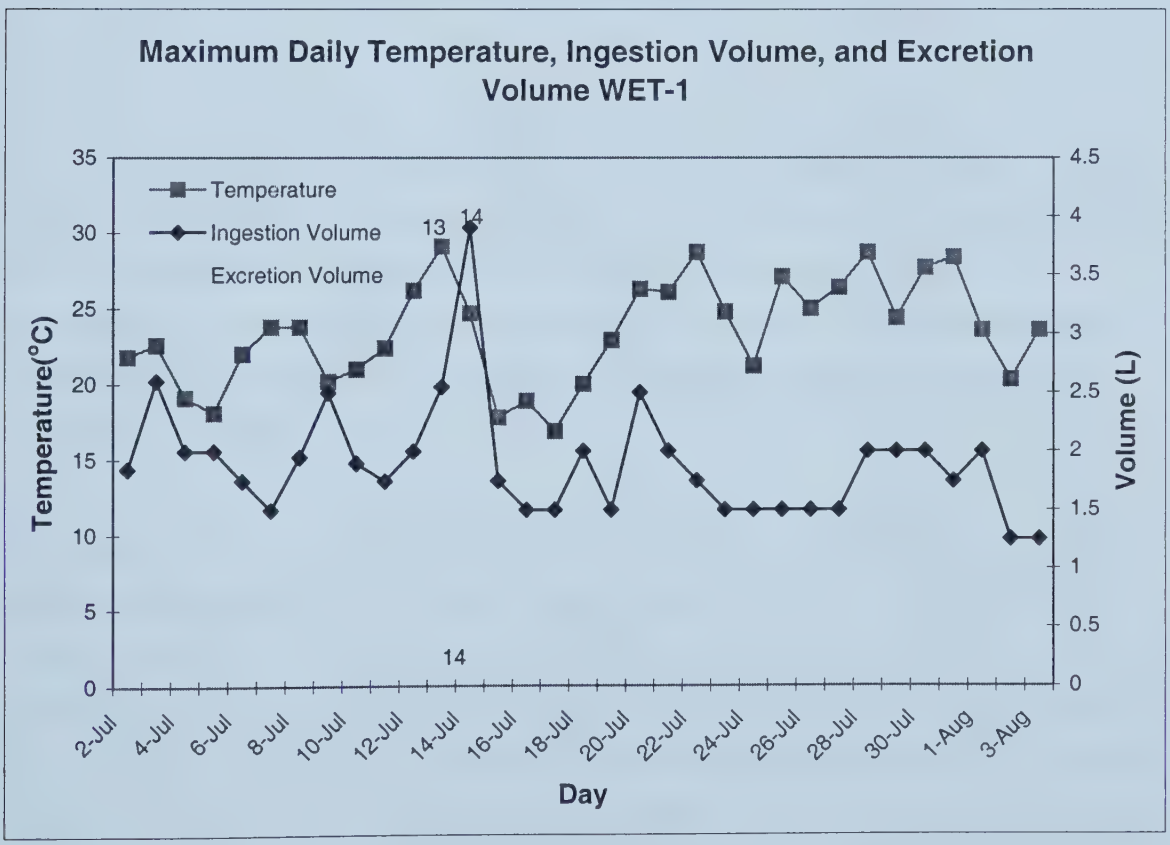


Figure II-6. Relationship between maximum daily temperature, ingestion volume, and excretion volume for participant WET-1 for all days of the study.

exposure from swimming possibly explains the high TCAA value for day two of the bottled water intervention. The high TCAA value for day eight of the bottled water intervention may also be explained by exposure to TCAA from swimming because participant WET-3 also recorded swimming two days previous to this study day.

The fact that participant WET-3 swam throughout the study, whereas no other volunteers recorded swimming, is also a possible explanation for the slightly longer half-life and poorer data fit for participant WET-3 as compared to the other participants. From Figures II-2 to 5, the TCAA elimination results for the last day of the bottled water intervention show that for participants WET-2, WET-4, and WET-5 (who followed the same study design as participant WET-3), creatinine adjusted TCAA excretion levels were all below 4.0 μg/g. Conversely, the final creatinine adjusted TCAA concentration for participant WET-3 was 13.7 μg/g. Furthermore, the starting creatinine adjusted TCAA concentrations for all of the other participants were higher than for WET-3, showing that the urinary concentrations of TCAA for WET-3 did not fall in a pattern similar to the other participants. Study participants were asked to record any beverages other than water that they consumed and analysis of the beverage consumption records for WET-3 did not show any consumption of fluids other than water. Thus, the increased TCAA elimination half-life and poorer fit for WET-3 are possibly due to TCAA exposures from swimming during the bottled water intervention. This outcome further illustrates the importance of accounting for all TCAA exposures when assessing the validity of an exposure biomarker.

The range of half-life values determined in this study (2.1 to 6.3 d) indicates that the elimination half-life of TCAA is significantly greater than the time interval between successive exposures to TCAA from periodic daily consumption. Because the elimination half-life is several times the expected interval between repeated exposures, TCAA will accumulate in plasma towards a quasi-steady state concentration. Assuming that urine concentrations parallel plasma concentrations, the urinary excretion of TCAA would reflect average exposure or dose over several days. Therefore, TCAA in urine appears to be persistent enough in the human body to provide a valid exposure biomarker for ingested TCAA from chlorinated drinking water for a reasonable exposure window.

There is limited literature documenting the pharmacokinetics of human TCAA elimination when TCAA is administered directly because most literature examines the behavior of TCAA as a metabolite of either CH or TCE. Based on ingesting between 6.25 and 40 mg/kg of CH, the half-life of TCAA measured in plasma ranges from 2.9-5.0 d (Breimer et al. 1974; Humbert et al. 1994). For TCAA as a metabolite of TCE, Bruning et al. (1998) cited an elimination half-life of 4.2 d but failed to provide experimental evidence or a reference for this value. Another study that measured the half-life of TCAA as a metabolite of TCE showed a 2.1 to 6.9 d half-life for TCAA (Yoshida et al. 1996), which is consistent with the 4.2 d half-life determined by Bruning et al. (1998).

The majority of the published half-life values for TCAA when TCAA is administered as the parent compound are for substantially higher doses of TCAA than occur in drinking water. Given an oral dose of 3 mg/kg of TCAA, Muller et al. (1972, 1974) reported an elimination half-life of 2.1 d. Earlier data from 3 volunteers showed elimination half-life values of 3.1, 3.2, and 4.1 d when they were given intravenous doses of TCAA of 28.1, 60.2, and 37.3 mg/kg, respectively (Paykoc and Powell 1945). The only elimination half-life values available for TCAA in drinking water are 2.3-3.7 d (Froese et al. Submitted). These results are consistent with our findings of a human TCAA half-life that ranges from 2.1 to 6.3 d. Also, this range indicates that there is substantial inter-individual variation in TCAA metabolism and excretion. Other investigators have observed similar inter-individual variability in TCAA metabolism and excretion (Paykoc and Powell 1945, Monster et al. 1979, Fisher et al. 1998). Monster et al. calculated the mean co-efficient of inter-individual variation in the amount of TCAA in urine over 24 hours to be 32 percent, using data from human volunteers.

Because this study involved both male and female volunteers and the volunteers were of varying ages, correlations between half-life and both gender and age can be made, albeit with a limited number of data points. Half-life values determined for the female volunteers showed an average of 2.3 d while the average of the half-life values for the male participants was 4.5 d. Of the available literature on the pharmacokinetics of TCAA, only Muller et al. (1974) reports the TCAA elimination half-life specific to

gender. They indicate that the average half-life for males (aged 20 to 30 years) is 2.1 d, which does not comply with the gender difference in half-life determined from this study. A possible difference is the age range of the male subjects in the Muller et al. (1974) study and this study. The age range of the male participants in the current study was 27 to 52 years, therefore, to evaluate if the higher average half-life value determined for males is a function of age, a correlation between age and half-life was also made (Figure II-7). This correlation may indicate that age is not a useful predictor of half-life and that the observed inter-individual variation in half-life may be influenced by gender. The limited number of data points used for suggesting this relationship and the influence of the long half-life for participant WET-3 limits making a meaningful correlation from this data.

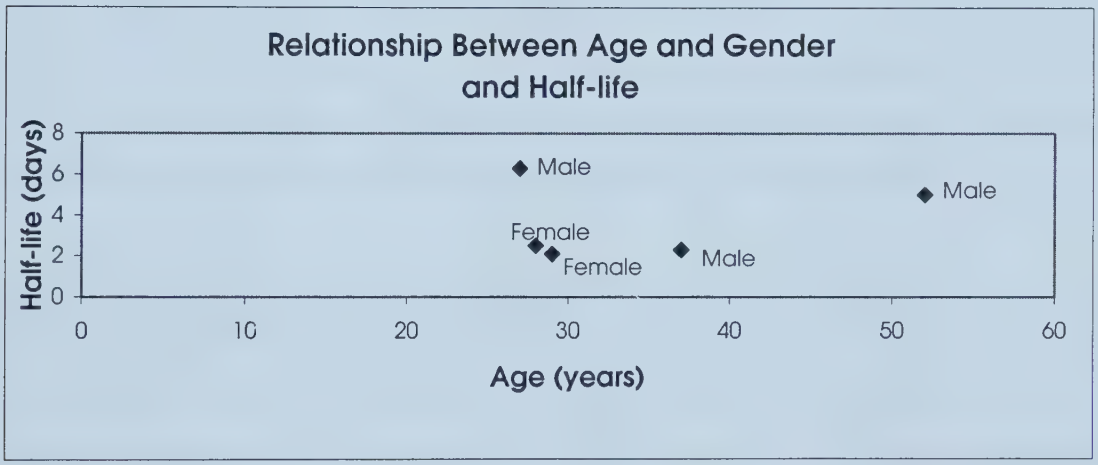


Figure II-7. Relationship between age and gender and half-life for all participants.

There is more confidence in the half-life values determined from this study as compared to the previous pilot study by Froese et al. because the values were calculated from more data points and have higher corresponding $R^2$ values. The half-life values determined from the Froese et al. (Submitted) study were 2.3, 2.9, and 3.7 d with coefficients of determination of 0.74, 0.70, and 0.45, respectively. All of the $R^2$ values for this study are better that the $R^2$ values determined from the previous pilot study (for a fewer number of data points) and even the one low value is nearly equal to the best coefficient of determination shown in the former study. These more consistent results in the current study are to be expected given the greater degree of control exerted over the individual TCAA exposures.

Each of the elimination half-life values from the previous pilot study was constructed from a plot of seven data points. Based on five degrees of freedom (df = n-2) the $R^2$ values for the half-lives of 2.3 and 2.9 d are significant at $p<0.05$ whereas the $R^2$ value for the 3.7 d half-life is not significant at $p<0.05$. The half-life values in this study were constructed from 20, 14, 14, 13, and 14 data points for participant WET-1, WET-2, WET-3, WET-4, and WET-5, respectively. The difference in the number of data points for WET-1 is that this participant started the study one day earlier than the other participants and remained on the bottled water intervention for a week longer than the other participants but was unable to provide a FMU sample on two days. Participant WET-4 was not able to provide a FMU sample on one day and, therefore, has one fewer data points than the other three participants who were also on bottled water for two weeks. All $R^2$ values determined for the half-lives in this study were significant at $p<0.001$. Moreover, all of the $R^2$ values except the $R^2$ value determined for the half-life of WET-3, were significant at $p<0.0005$. Thus, there is more confidence in the half-life values determined from this study as compared to the previous pilot study.

The quality of the half-life values determined from this study was likely achieved by the TCAA ingestion being well controlled and documented. For this study, participants were asked to refrain from drinking any beverages other than the water provided (with the exception of milk) to limit TCAA ingestion sources that could not be directly analyzed for TCAA. Beverage type and ingestion volume were allowed to follow normal behavior in the former study (Froese et al. Submitted). As a result, there is more intra-individual variability in the daily excretion values for TCAA and there is TCAA ingestion that cannot be accounted for as a result of ingestion of other beverages.

*Elimination Half-life Estimation Based on TCAA Elimination Rate*

To determine the feasibility of using TCAA excretion rate as an alternative to creatinine-normalized concentrations in half-life determinations, elimination half-life values were also determined using urinary TCAA excretion rate. For these elimination half-life estimates, the TCAA concentrations measured in FMU samples were adjusted for volume and time according to Equation (3). This TCAA elimination rate was plotted against time

in days, with day zero being the average of all days on Winnipeg water and day one being the first day of the bottled water intervention. The logarithm of the TCAA urinary excretion rate was evaluated for linear fit against time (corresponding to exponential decay) and half-life values were determined in a method analogous to that used for the creatinine adjusted TCAA elimination half-life estimations.

$$\text{TCAA excretion rate } \left(\frac{\mu g}{d}\right) = \text{TCAA concentration } \left(\frac{\mu g}{L}\right) \text{ x Volume (L)} \text{ x Time interval } \left(\frac{1}{hr}\right) \text{ x Day correction } \left(\frac{24\ hr}{d}\right) \quad (3)$$

Half-life values for the five participants, as determined from the FMU TCAA elimination rate versus time curves were 2.2, 2.2, 2.2, 4.6, and 5.3 d with $R^2$ values of 0.70 (n=14), 0.72 (n=14), 0.94 (n=13), 0.84 (n=20), and 0.62 (n=14), respectively (Figures II-8 to 12). In comparison to the elimination half-life values determined using the creatinine adjusted TCAA concentrations, the elimination half-lives determined from TCAA excretion rate are not substantially different. One exception is the half-life determined for WET-3, which differs by one full day. Table II-2 provides a summary of the elimination half-life and corresponding $R^2$ values for both the creatinine adjusted TCAA and TCAA excretion rate half-life determinations. Except for participants WET-1 and WET-4, whose $R^2$ values increased slightly and stayed the same, respectively, all of the $R^2$ values for the rate method for the other three participants decreased relative to the creatinine adjusted method.

The half-life curve for participant WET-1 appears more consistent for the excretion rate elimination method (Figure II-8) than for the creatinine adjusted TCAA method (Figure II-1) because of the more dramatic outlier for day two of the bottled water intervention using the creatinine method. The FMU sample for participant WET-1 for that day was low in creatinine and high in volume relative to the TCAA concentration making the creatinine adjusted TCAA concentration seem higher in relation to the TCAA excretion rate.

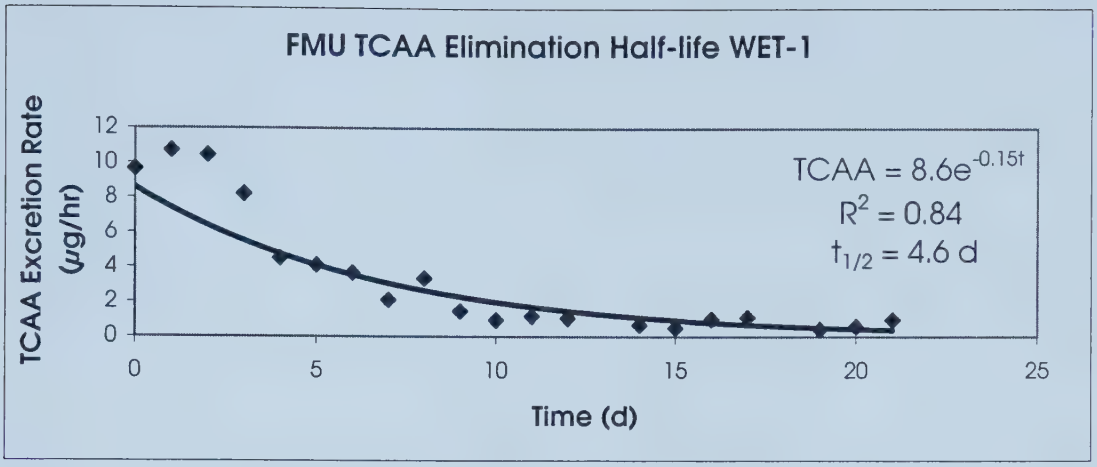


Figure II-8. TCAA elimination half-life estimation for WET-1 using TCAA elimination rate from FMU excretion.

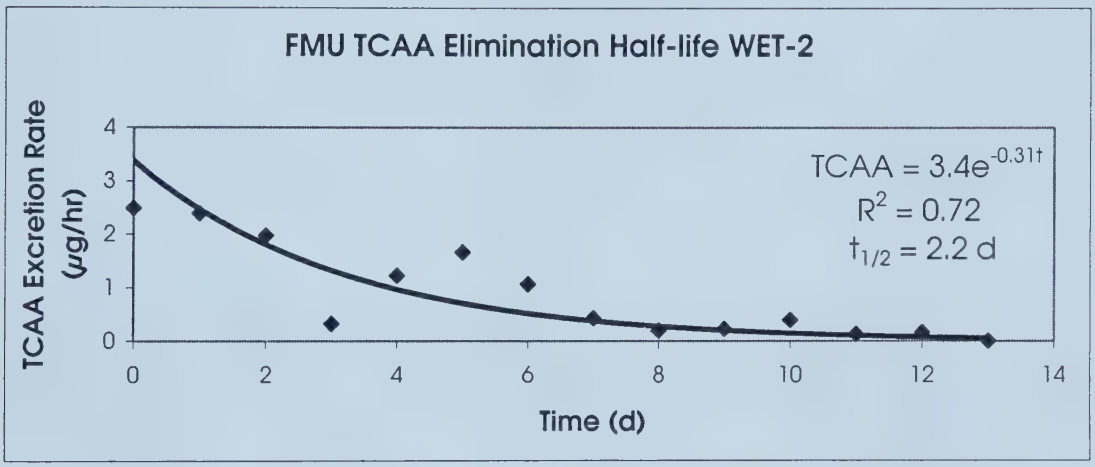


Figure II-9. TCAA elimination half-life estimation for WET-2 using TCAA elimination rate from FMU excretion.

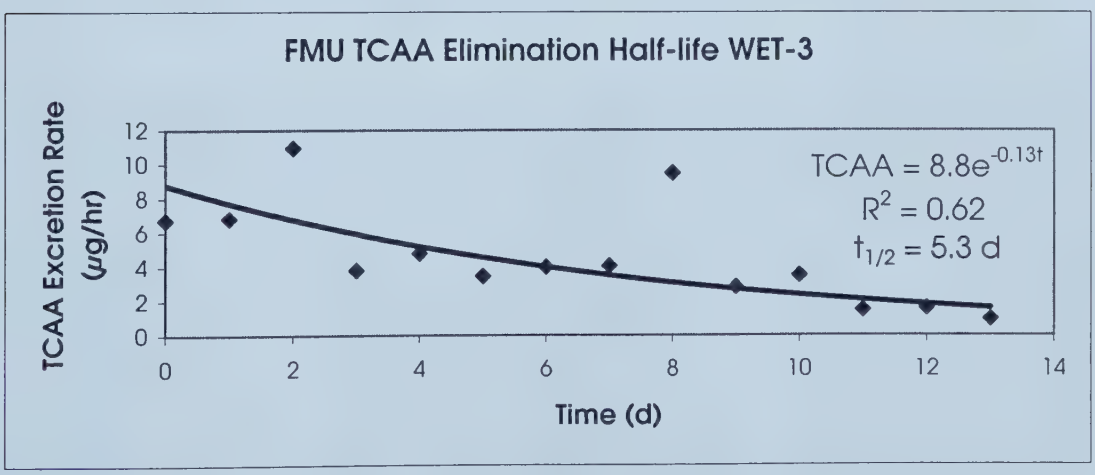


Figure II-10. TCAA elimination half-life estimation for WET-3 using TCAA elimination rate from FMU excretion.

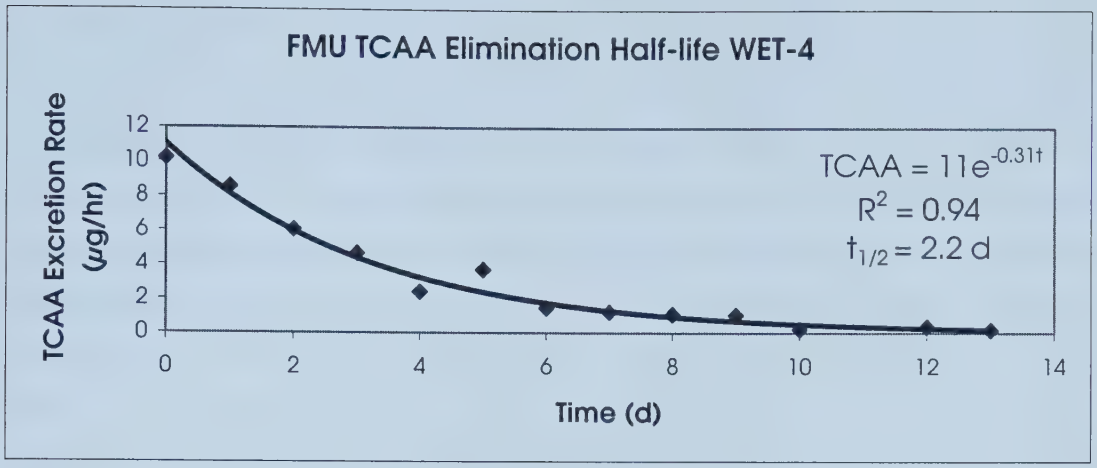


Figure II-11. TCAA elimination half-life estimation for WET-4 using TCAA elimination rate from FMU excretion.

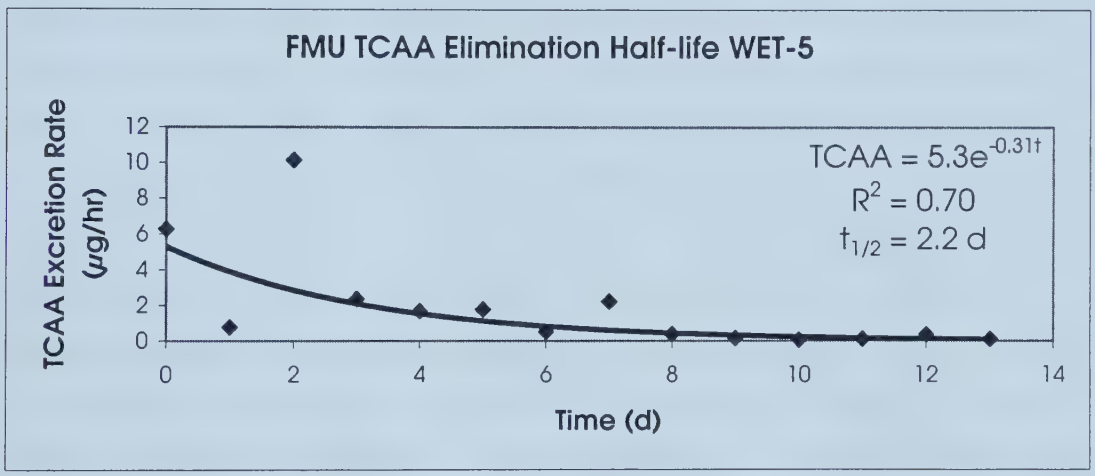


Figure II-12. TCAA elimination half-life estimation for WET-4 using TCAA elimination rate from FMU excretion.

Table II-2. Half-life values and coefficients of determination for the creatinine adjusted TCAA and TCAA excretion rate elimination half-life calculations.

| | Creatinine Adjusted TCAA | | TCAA Excretion Rate | |
|---|---|---|---|---|
| **Participant** | **Half-life (d)** | **Coefficient of Determination** | **Half-life (d)** | **Coefficient of Determination** |
| WET-1 | 5.0 | 0.80 | 4.6 | 0.84 |
| WET-2 | 2.1 | 0.83 | 2.2 | 0.72 |
| WET-3 | 6.3 | 0.76 | 5.3 | 0.82 |
| WET-4 | 2.3 | 0.94 | 2.2 | 0.94 |
| WET-5 | 2.5 | 0.88 | 2.2 | 0.70 |

For participants WET-2, 3, and 5 the $R^2$ values that correspond to the half-lives are significant at a weaker level than the $R^2$ values for the half-lives determined from the creatinine adjusted TCAA elimination. The $R^2$ values for the half-lives for WET- 2 and WET-5 based on TCAA excretion rate are significant at $p<0.005$, as compared to the $R^2$ values for the creatinine adjusted TCAA elimination half-lives that were determined to be significant at $p<0.0005$. Likewise, the $R^2$ values for the half-life for WET-3 based on creatinine adjusted TCAA was significant at $p<0.005$, whereas for TCAA excretion rate the $R^2$ for the half-life was only significant at $p<0.025$. Therefore, using creatinine adjusted TCAA appears to be a more accurate method for determining TCAA elimination half-life.

Estimation of TCAA elimination half-life from its urinary excretion rate depends on knowing both the urine volume and the time interval between successive urination episodes. Requiring study subjects to document urination times demands more of their participation and is subject to errors in remembering and recording. Measuring urine volume and urination times are not only subject to practical and logistic limitations but are also subject to exogenous influences that are difficult to control. Urine volumes fluctuate considerably in response to changes in fluid consumption, physical exercise, and environmental temperature. These fluctuations occur both between individuals (inter-individually) and in the same individual at different times (intra-individually). Ideally, urinary rates per day would be based on true 24-hour urine samples, however, these samples are inconvenient for the participant to collect. Also, the sample may not truly represent the time interval intended due to differences in bladder emptying at the beginning and the end of the time interval (Jackson 1966).

Creatinine is a waste product of normal muscle metabolism and is infused into the general circulation by muscle at a nearly constant rate. Thereafter, creatinine is removed from plasma primarily by glomerular filtration in the kidneys and is eliminated from the body in urine. Ambient temperature, exercise, fluid consumption, or diet does not largely influence the amount of creatinine excreted. Although creatine, the metabolic precursor of creatinine, is present in lean meats, normal diets contain only a trace amount of

creatinine (Jackson 1966). Since creatinine is derived almost entirely from endogenous metabolism and is not resorbed by the renal tubules, urinary creatinine excretion is a relatively steady process. Therefore, creatinine-normalized TCAA concentrations can be used reliably to adjust for variations in urine volume. Circadian rhythms in the amount of creatinine excreted in urine have been reported (Lakatua et al. 1982). However, because the FMU samples were collected at approximately the same time every day it is unlikely that a circadian rhythm in urinary creatinine excretion influenced the creatinine adjusted TCAA results. All-day urine collection by one participant allowed determination of the variation of creatinine excretion in FMU as compared to the rest of the day. For this one participant the creatinine excreted in FMU samples (average = 1.9 g/d, standard deviation = 0.3 g/d) nearly equaled the creatinine excreted in urine samples for the remainder of the day (average = 1.8 g/d, standard deviation = 0.3 g/d) showing that any diurnal variation in creatinine excreted in urine probably did not influence the creatinine adjusted TCAA results. Because creatinine is subject to fewer influences than urine volume or time interval it is explainable that using creatinine adjusted TCAA to determine TCAA elimination half-life is a more accurate method than adjusting TCAA in urine for time since last urination and urine volume.

*FMU as a Representative of Average Daily TCAA Excretion*

For the one participant who collected every urine sample for every day of the study, the elimination half-life calculated from creatinine adjusted FMU TCAA excretion only (5.0 d, $R^2$=0.80) (Figure II-1) was not different from the half-life calculation based on the daily average of total daily creatinine adjusted TCAA excretion (5.0 d, $R^2$=0.85) (Figure II-13), except that the coefficients of determination were slightly different. On average, the contribution of TCAA from FMU to the total daily TCAA excretion was 0.38 (range 0.18-0.56) (Figure II-14). Likewise, the median also equaled 0.38, showing that there is an equal number of data points on both sides of the average and that the average is not skewed by a disproportionately high or low value. This indicates that FMU appears to be representative of daily TCAA excretion. However, it is important to note that these data are from one participant only and do not represent an average of all participants. Further, the consistency observed in the proportion of total daily TCAA excreted in FMU is likely

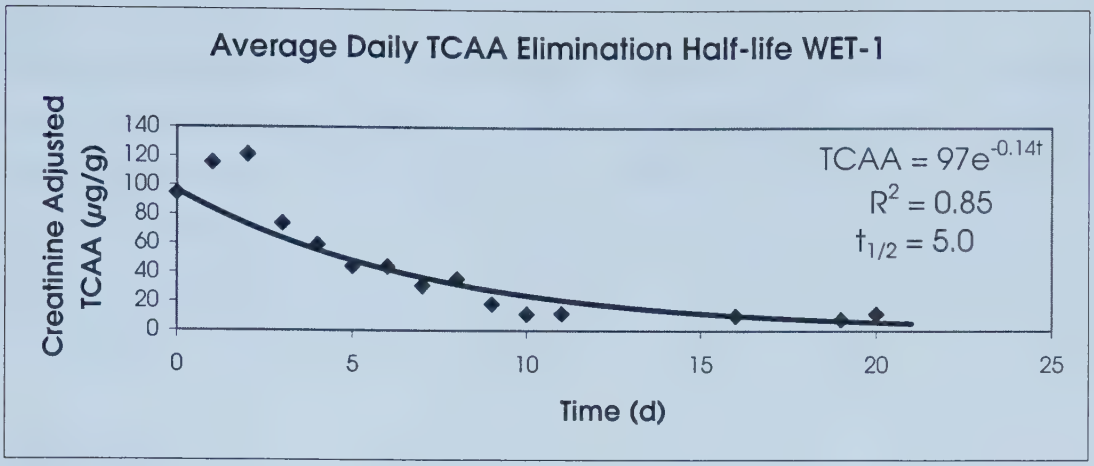


Figure II-13. TCAA elimination half-life for WET-1 using average daily TCAA excretion.

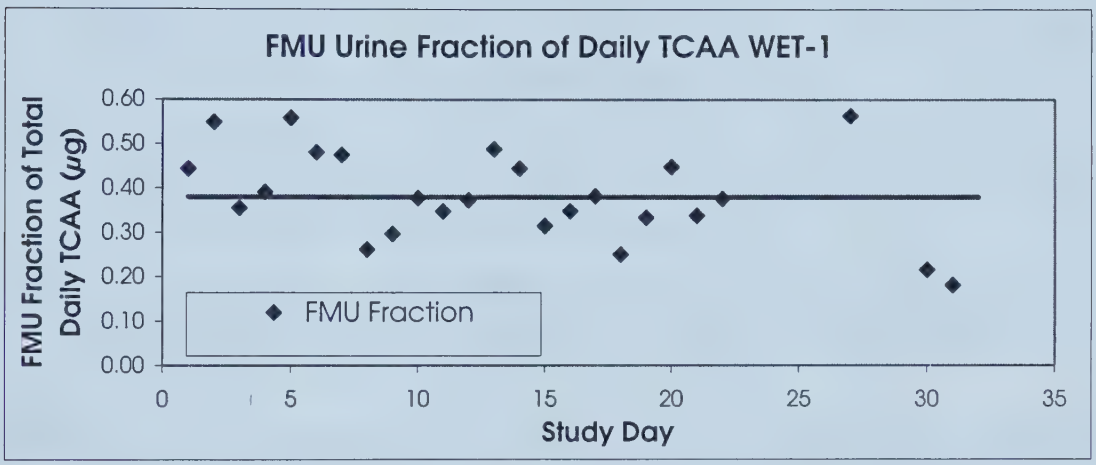


Figure II-14. The FMU fraction of average daily TCAA excretion for WET-1.

dictated by asking participants to consume a minimum of 1000-mL of water after 17:00 hr each day. Additional research on this topic is warranted.

### *Conclusions*

Overall, these results allow for improved confidence in the validity of using TCAA as a biomarker for DBP exposure. TCAA, as indicated by the half-lives measured, appears to be persistent enough in the human body to be viable as a biomarker of exposure when TCAA is administered at drinking water concentrations. However, factors that contribute to inter-individual variability in TCAA metabolism and excretion need to be identified. Creatinine adjusted TCAA elimination appears to be an equivalent and perhaps more accurate method of determining TCAA elimination than using TCAA elimination rate.

Eliminating the requirement for participants to record the time interval over which a urine sample was collected shows increased feasibility of applying this biomarker method to epidemiologic studies. The potential for using FMU as an accurate representative of daily TCAA excretion also improves the feasibility of using TCAA as a biomarker of exposure in epidemiologic studies because FMU sampling is relatively unobtrusive and does not require a great deal of time and effort to collect.

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# III TCAA EXCRETION IN RELATION TO INGESTION EXPOSURE

## *Introduction*

Exposure to disinfection by-products (DBPs), compounds formed as a consequence of disinfecting water for human use (Bellar et al. 1974; Rook 1974), has come under increasing scrutiny because of the suspicion that DBPs cause adverse reproductive health effects (Kramer et al. 1992; Bove et al. 1995; Gallagher et al. 1998; Dodds et al. 1999; King et al. 2000; Savitz et al. 1995; Swan et al. 1998; Waller et al. 1998). Understanding of the relationship between exposure to DBPs and adverse reproductive effects is limited, however, by inaccurate individual-level exposure assessment in epidemiologic studies. In terms of bioavailability, DBP concentrations in chlorinated water are distinguished as the *external exposure*, that amount of compound that is absorbed (ingested, inhaled, or absorbed through the skin) is defined as the *external dose*, and the dose of DBPs that reaches the systemic circulation is the *internal dose* (Hrudey et al. 1996). On the continuum external exposure-external dose-internal dose, internal dose is the most individualized measure of exposure and, therefore, the most desirable measure of exposure to DBPs.

The majority of the current epidemiologic studies have assessed exposure by assigning routinely collected DBP concentrations measured at water treatment facilities to women based on the water facility that served her residence during the time of her pregnancy (Kramer et al. 1992; Bove et al. 1995; Gallagher et al. 1998; Dodds et al. 1999; King et al. 2000). This represents a measure of external exposure to DBPs. Improvements to exposure assessment have been achieved by combining information on water consumption practices with routinely collected DBP concentrations to get an approximate measure of external dose (Savitz et al. 1995; Swan et al. 1998; Waller et al. 1998). None of these epidemiologic studies, however, has used true individual monitoring for exposure assessment.

Humans are exposed to DBPs by three routes – ingestion, inhalation, and dermal absorption. The contribution of each exposure route to the overall exposure to DBPs

from chlorinated water depends on the water practices of the individual and the respective physiochemical properties of individual DBPs (Weisel et al. 1999). Ignoring or generalizing the ways and amounts in which water is used introduces uncertainty into the epidemiologic method, reducing confidence that the risks measured using such methods are the true reproductive risks of exposure to DBPs (Reif et al. 2000).

Given the need for individual exposure assessment and the complexities of exposure to DBPs, a biomarker of exposure is a desired possibility for improving exposure assessment in epidemiologic studies of the relationship between DBPs and adverse reproductive outcomes. Biomarkers of exposure are parent compounds or their metabolites that show a quantifiable relationship to the amount of compound to which an individual was exposed. Using the foregoing bioavailability terminology, biomarkers of exposure represent measures of internal dose. Thus, if DBPs or their respective metabolites can be measured in human biological media (blood, urine, breath, sweat, saliva) and these measurements correlate to levels of DBPs in drinking water there is potential for exposure biomarkers to provide individual exposure assessment in epidemiologic studies.

Of the compounds that have been investigated as potential biomarkers of exposure for DBPs, trichloroacetic acid (TCAA) in urine has shown the most promise. Weisel et al. (1999) and Kim et al. (1999), in a cross-sectional study of 49 women, found higher urinary excretion levels of TCAA for higher exposure groups as compared to those groups exposed to lower levels of TCAA. The cross-sectional design, however, precluded judgements on TCAA excretion time trends and intra- and inter-individual variability of TCAA ingestion and excretion. Also, ingestion was assessed retrospectively and was quantified only from tap water consumed at home and did not take into account TCAA ingestion from water sources outside the home or from beverages other than water.

To assess the temporal relationship between TCAA ingestion and excretion, Froese et al. (Submitted) conducted a longitudinal pilot study in Adelaide, Australia using 10 human participants. Ingestion exposure was assessed prospectively with consumption diaries

and tap water analyses for an initial period while participants consumed their normal tap water supply, a subsequent bottled water intervention, and a final return to normal tap water. Volunteers were not asked to limit beverage consumption to only water. Excretion of TCAA was determined by analysis of urine samples for selected study days. Results of this pilot study showed substantial intra-individual variability for both ingestion (14% to 67% relative standard deviation [SD%]) and excretion (15 – 71 SD%) of TCAA. Likewise, wide variability was also shown for inter-individual TCAA ingestion (54 – 100 SD%) and excretion (54 – 150 SD%). Large fluctuations in water volume consumed, TCAA ingestion from beverages other than water, and daily variations in TCAA concentrations measured in tap water were cited as possible explanations for the variability in TCAA ingestion and excretion. Comparing measured TCAA excretion and a calculated upper bound for TCAA excretion for two participants using the TCAA elimination half-lives determined for those participants, higher TCAA ingestion showed a reasonable correlation with high TCAA excretion ($R=0.74, 0.84$; $P<0.005$ for $n=10$). Similarly, for two specified days of the study, higher TCAA ingestion (adjusted back for three days using half-life) for all participants correlated well with higher TCAA excretion ($R=0.88, 0.90$; $P<0.005$ for $n=9$) (Froese et al. Submitted).

Given the favorable results of the Froese et al. pilot study, this longitudinal pilot study was undertaken to further determine the temporal trends of TCAA ingestion and excretion and improve understanding of TCAA intake sources and volumes. In an effort to reduce the intra- and inter-individual variability of TCAA ingestion and excretion, beverage consumption was limited to water of known TCAA concentration and ingestion volumes were managed. This study also evaluates how well TCAA ingestion correlates with biomarker concentrations of urinary TCAA to determine the validity of using TCAA as a biomarker of exposure for epidemiologic investigation.

## *Methods*

### *Volunteer Recruitment*

Five participants were recruited from the University of Alberta in Edmonton, Alberta, Canada for participation in the study. Of the five volunteers, three were male (age range 27 – 52 years) and two were female (age range 28 – 29 years). All subjects were either students or staff from our research department. Therefore, these volunteers were aware of the need for improved exposure biomarker research for DBPs and were highly respectful of maintaining the study design.

### *Water Source*

Drinking water in Edmonton contains very low levels of TCAA (Epcor 2000). Because the results of the earlier pilot study conducted by Froese et al. (Submitted) showed the importance of having sufficiently high TCAA ingestion exposures to obtain sufficiently high measures of TCAA excretion, water was imported from Winnipeg, Manitoba, Canada for use in Edmonton. The higher TCAA concentrations in Winnipeg water than in Edmonton water are a function of different chlorination methods and chlorine contact times.

For this study, it was assumed that ingestion is the primary exposure route for TCAA because TCAA is nonvolatile, therefore, having minimal inhalation. Also, because TCAA is polar it has low skin permeability and minimal dermal absorption from routine household uses (Kim and Weisel 1998). Another advantage of Edmonton drinking water having substantially lower TCAA levels than Winnipeg drinking water is that inhalation and dermal TCAA exposures would be less likely to influence the ingestion results.

Two separate shipments of water were sent from Winnipeg on two different dates. Regardless of shipment date, all water was collected from the same location in Winnipeg. Water was shipped and stored in 20-L polycarbonate Nalgene carboys. Volunteers were

also provided with smaller plastic containers (Rubbermaid™) for storage and transport of smaller water volumes and were given water as it was needed.

*Study Design*

This study was designed as a longitudinal pilot exposure/intervention study. The exposure period involved participants consuming normal tap water from Winnipeg for two weeks and then switching to TCAA-free bottled water consumption for a two-week intervention period. One participant remained on bottled water for three weeks. The TCAA-free bottled water was obtained from Sierra Spring Water Company in Edmonton. First morning urine (FMU) samples were obtained from all volunteers for every day of both the exposure and intervention periods. One volunteer collected all urine samples throughout the entire day for both study periods and recorded all urination times. A summary of the study design is outlined in Table II-1 (Chapter II). All data were collected between 3 July 2000 and 4 August 2000.

To eliminate the influence of TCAA ingestion from unknown sources, participants were asked to limit their beverage consumption to the water provided. Milk consumption was allowed as milk is not expected to contain TCAA and is an important calcium source. To manage ingestion volume, as would be reflected in the FMU samples, participants were asked to consume at least 1000-mL of water after 17:00 hr for each day of the study. Dedicated measuring cups were provided to each volunteer to directly measure water consumption volumes.

Each subject was provided with one daily diary for each week of the study. In this diary, the participant prospectively recorded the collection times of the urine samples, previous urination times, the time and amount of all fluid consumption (including beverages other than the water provided, if consumed), and the time and duration of specified water-related activities. Water-related activities assessed were showering, bathing, bathing others, washing dishes or cars, swimming, streamroom/sauna use, and hot-tub use. Participants were also asked to document the time and amount of any medications taken in case of potential interference. For example, chloral hydrate, a compound of which

TCAA is a metabolite, is used medicinally. The diaries also included a space to record any visits to dry-cleaning facilities because these might involve exposure to trichloroethylene (TCE). Winnipeg water consumption and diary documentation was started 48 hr prior to the first day of urine sample collection. In the analyses the first day of urine collection was considered as the first study day.

*Urine Samples*

Entire FMU voids were collected in 1000-mL high-density polyethylene bottles and were brought to the laboratory by the volunteers for analysis. If a delay of more than four hours was expected between sample collection and delivery to the lab, the samples were stored in a refrigerator or in a cooler with ice. Once in the lab, urine samples were stored in a refrigerator (4°C) until analysis. All samples were extracted within 24 hr of collection.

In the laboratory, sample volume was measured and 10-mL of each sample was poured off into a polyethylene vial. This 10-mL sample was transported to Dynacare Kasper Medical Laboratory (DKML) in Edmonton for creatinine analysis. DKML used a colorimetric method to analyze creatinine that involved the reaction of creatinine with sodium picrate and UV absorption at 500 nm (DKML 2000). Creatine analysis was performed to correct for variations in urine volume (Jackson 1966). Creatine, the metabolic precursor of creatinine, is available commercially for use as an exercise supplement, therefore, all participants were assessed for creatine use before the study began. No participant reported using supplemental creatine.

The amount of TCAA excreted in urine was analyzed using a modified version of EPA Method 552.2 (USEPA 1995) that employed the use of solid phase microextracton (SPME) (Sarrion et al. 1999). Using a graduated measuring cylinder, 40-mL of urine was measured and poured into a 50-mL centrifuge tube. A surrogate standard made up of 75-µL of a 25 µg/mL solution of 2,3-dibromopropionic acid was added to each sample. Subsequently, 2-mL of concentrated sulfuric acid (Fisher Scientific), approximately 12-g of sodium sulfate (Fisher Scientific), and 4-mL of methyl tert-butyl ether (MTBE) (PRA

Grade, Aldrich) were added and the samples were shaken on a mechanical shaker (Vibrax-VXR) for nine minutes. After shaking, the samples were centrifuged at 2500 rpm for five minutes (Sorvall Biofuge Primo, Mandel Scientific Company).

The entire solvent layer was removed from the top of the sample using a glass Pasteur pipette, placed in a 10-mL glass vial, and evaporated with nitrogen gas (UHP grade) using an evaporator (Pierce Reacti-Vap 18780) while being heated at 50°C (Pierce Reacti-Therm). The TCAA in the samples was derivatized to its methyl esters using 53-µL of methanol (HPLC Grade, Fisher Scientific) and 97-µL of concentrated sulfuric acid (Fisher Scientific) and the vials were immediately sealed with crimp-tops. After heating the sample for 10 minutes at 50°C, the SPME fiber (polydimethylsiloxane (PDMS), 100-µm thickness) was exposed to the headspace and the TCAA methyl esters were allowed to absorb to the fiber for 10 minutes. The fiber was desorbed in the injection port of the gas chromatograph (GC) for two minutes at 300°C. All headspace sampling and injection was achieved using an auto-sampler (Varian 8200). Analyses were performed on a GC equipped with an electron capture detector (ECD) (Varian 3800). A 30 m x 0.25 mm i.d., 0.25 µm film thickness DB-5 (5% diphenyl and 95% dimethylpolysiloxane) column (J and W Scientific Co.) was used. The column temperature program was 40°C (held for 10 minutes) to 75°C (held for five minutes) at 5°C per minute and up to 150°C (held for one minute) at a rate of 20° per minute. The inlet and detector temperatures were maintained at 300°C and 350°C, respectively, the GC was operated in splitless mode, and a 95% argon/5% methane mix was used as the detector make-up gas (flow rate 28 m per minute). Calibration of the GC was done with a mixture of methyl ester HAAs (Supelco) (calibration range 1 to 200 µg/L for TCAA). The linear calibration curve used for the urine samples had an $R^2$ value of 0.9992 (Appendix 1).

*Water Samples*

Every day that the participants consumed the Winnipeg tap water, samples of the tap water were analyzed using an analogous method to the urine samples. One exception to this was that the water samples were not centrifuged. The Winnipeg tap water analyzed for TCAA was stored unpreserved in a polycarbonate Nalgene carboy identical to the

carboys used by the participants. This water was kept in a refrigerator (4°C) to simulate the conditions under which participants stored their supplies of drinking water.

***Results and Discussion***

*TCAA Ingestion*

The method used for analyzing TCAA concentrations in water samples showed sensitive detection of TCAA (Method Detection Limit [MDL] = 0.82 μg/L) and good repeatability (relative standard deviation of 8.7% on 3 triplicate analyses of water). Method detection limit was calculated as three times the standard deviation of seven water samples of very low concentration (Taylor 1987). The bottled water was confirmed TCAA-free by analysis as the average of seven replicates (0.14 μg/L) was below the MDL. The amount of TCAA ingested (TCAA-ing) by each participant per day was calculated by multiplying the TCAA concentration measured in the water sample for a specified day by the amount of water that the participant consumed on the corresponding day according to equation 1. One hundred percent absorption across the gastrointestinal tract was assumed so that the TCAA-ing is presumed to be equivalent to the exposure.

$$\text{TCAA - ing}\left(\frac{\mu g}{d}\right) = \text{TCAA concentration in water}\left(\frac{\mu g}{L}\right) \text{x Volume consumed (L)} \\ \text{x Time interval}\left(\frac{1}{hr}\right) \text{x Day correction}\left(\frac{24\ hr}{d}\right) \quad (1)$$

Table III-1 shows the intra-individual variability of TCAA-ing based on 13 days of tap water ingestion. These data were collected from 2 July 2000 to 14 July 2000 for participant WET-1 and from 3 July 2000 to 15 July 2000 for participants WET-2, 3, 4, and 5. The relative standard deviation of TCAA ingestion for the five participants had a small range of 43% to 52% and an average relative standard deviation of 46%. Results from the previous pilot study, in which ingestion sources and volumes were not managed, indicated a substantially greater range of intra-individual TCAA ingestion (14% to 67%) (Froese et al. Submitted). The findings from this study suggest that when ingestion

Table III-1. Intra-individual variability of TCAA ingestion for all participants based on 13 days of tap water ingestion.

| Participant | Average Ingestion (μg/d) | Standard Deviation | Relative Standard Deviation | Minimum Ingestion (μg/d) | Maximum Ingestion (μg/d) |
|---|---|---|---|---|---|
| WET-1 | 270 | 140 | 52% | 150 | 610 |
| WET-2 | 220 | 99 | 45% | 120 | 340 |
| WET-3 | 440 | 190 | 43% | 250 | 780 |
| WET-4 | 300 | 140 | 47% | 150 | 540 |
| WET-5 | 350 | 150 | 43% | 210 | 670 |
| Range | 220 – 440 | 99 – 190 | 43% – 52% | 120 – 250 | 340 – 780 |

volumes and beverage sources are managed, variation in TCAA ingestion between participants can be reduced but day-to-day differences in water consumption causes ingestion to vary by approximately 50%.

To evaluate the inter-individual variability in TCAA ingestion the differences in TCAA ingestion between all participants for all days of the study was calculated. These results are presented in Table III-2. The data show that the daily relative standard deviation among all of the participants ranged from 16% to 58% with an average of 33%. The low value of 16% relative standard deviation and the high value of 58% relative standard deviation appear to be outliers as without these two values the range in inter-individual ingestion variability is 25% to 38%, with an average of 32%. Analysis of the daily diaries for all of the participants shows ingestion volumes between participants were uncharacteristically similar for study day 2 in relation to the other study days, explaining the low relative standard deviation for day 2. The daily diaries also showed one participant consumed a particularly high volume of water on day 13 as compared to the other participants, which explains the high relative standard deviation for day 13 later explained as exercise and ambient temperature related.

Multiple linear regression was performed to evaluate the relative influence of water ingestion volume versus TCAA concentration on the absolute mass of TCAA ingested. For these analyses TCAA concentration and ingestion volumes for the second shipment of Winnipeg tap water were used to eliminate the influence of the substantial difference in TCAA concentration between the two shipments of water and the contribution of previous unmeasured TCAA ingestion prior to the onset of the study. Results of these analyses showed that for all but one participant (WET-2), ingestion volume contributed more than TCAA concentration to the absolute amount of TCAA ingested (Table III-3), as indicated by the magnitude of the standardized regression coefficient. Average daily ingestion volumes for WET-2 (1.5 L) were considerably lower than the average daily ingestion volumes of the other participants (1.9, 3.3, 2.3, 3.2 L for participants WET-1, 3, 4, and 5, respectively). These results suggest than when ingestion volumes are high, ingestion volume will contribute more to the absolute amount of ingested TCAA from

Table III-2. Inter-individual variability of TCAA ingestion for all participants based on 13 days of tap water ingestion.

| Study Day | Average Ingestion (µg/d) | Standard Deviation | Relative Standard Deviation | Minimum Ingestion (µg/d) | Maximum Ingestion (µg/d) |
|---|---|---|---|---|---|
| 1 | 440 | 110 | 25% | 270 | 530 |
| 2 | 360 | 57 | 16% | 310 | 450 |
| 3 | 220 | 63 | 29% | 150 | 290 |
| 4 | 200 | 63 | 32% | 130 | 290 |
| 5 | 200 | 63 | 32% | 130 | 290 |
| 6 | 190 | 56 | 29% | 120 | 250 |
| 7 | 220 | 84 | 38% | 150 | 350 |
| 8 | 220 | 68 | 31% | 150 | 300 |
| 9 | 200 | 64 | 32% | 160 | 300 |
| 10 | 530 | 180 | 34% | 360 | 750 |
| 11 | 470 | 150 | 32% | 240 | 620 |
| 12 | 460 | 160 | 35% | 270 | 640 |
| 13 | 430 | 250 | 58% | 210 | 780 |
| Range | 190 – 530 | 56 – 250 | 16% – 58% | 120 – 360 | 250 – 780 |

Table III-3. Results of multiple linear regression performed to evaluate the influence of TCAA concentration in tap water and tap water ingestion volume on the absolute amount of TCAA ingested[1].

| | Standardized Regression Coefficient (β) | |
|---|---|---|
| **Participant** | **Ingestion Volume** | **TCAA Concentration** |
| WET-1 | 1.2 | 0.22 |
| WET-2 | 0.086 | 0.96 |
| WET-3 | 0.98 | 0.033 |
| WET-4 | 0.82 | 0.27 |
| WET-5 | 0.92 | 0.12 |

1. Absolute amount of TCAA was used as the dependent variable and TCAA concentration and ingestion volumes were used as the independent variables.

chlorinated drinking water but in the case where ingestion volumes were lower the TCAA concentration had more influence over the amount of TCAA ingested.

The above outcomes indicate the importance of accurately ascertaining individual consumption volumes when determining individual exposures to DBPs in drinking water. Traditionally, epidemiologic methods that have assessed water consumption amounts have used average ingestion amounts for exposure assessment (Savitz et al. 1995; Swan et al. 1998; Waller et al. 1998). The one exposure biomarker study published for TCAA to date also did not precisely determine water ingestion volumes because retrospective assessment was used to quantify water consumption (Kim et al. 1999; Weisel et al. 1999). These methods, at best, determine approximate water ingestion volumes. Because fluctuating water volumes have been suggested here to be the largest influence on day-to-day ingestion of TCAA, average and retrospective ascertainment of water consumption are expected to introduce substantial uncertainty into the exposure assessment.

An unforeseen issue was the extent of decline (~ 50%) in the TCAA concentrations of Winnipeg tap water during the study. This trend was seen for both shipments of Winnipeg drinking water that were used (Table III-4). Subsequent analysis of water samples shipped from Winnipeg to Edmonton showed that the TCAA concentrations in unpreserved water stored in the plastic Nalgene containers fluctuated in a manner similar to the Winnipeg water used for the duration of the study. Specifically, these secondary results showed that the TCAA concentrations also declined by approximately 50%. Samples of the same water stored in amber glass bottles in the refrigerator and preserved

with ammonium chloride according to USEPA procedures (USEPA 1995) showed that the TCAA concentrations did not fluctuate under these ideal storage conditions (Table III-5). Concentrations of TCAA in urine stored in plastic bottles did not fluctuate, as did the water samples. Possible reasons for this discrepancy are the different plastics that were used for the water (polycarbonate) and urine (polypropylene or polyethylene), free chlorine not being present in the urine samples, or matrix differences between the urine and water. The fact that when water is unpreserved, TCAA concentrations fluctuate requires further investigation to understand how and why this occurs.

Table III-4. TCAA concentrations in both shipments of Winnipeg water for the duration of the exposure period for the exposure/intervention study[1].

| First Shipment of Winnipeg Water | | Second Shipment of Winnipeg Water | |
|---|---|---|---|
| **Date** | **TCAA Concentration (μg/L)** | **Date** | **TCAA Concentration (μg/L)** |
| 4 July 2000 | 97.1 | 12 July 2000 | 182.5 |
| 5 July 2000 | 52.9 | 13 July 2000 | 103.9 |
| 6 July 2000 | 50.4 | 14 July 2000 | 106.7 |
| 7 July 2000 | 50.6 | 15 July 2000 | 97.1 |
| 8 July 2000 | 47.6 | | |
| 9 July 2000 | 50.1 | | |
| 10 July 2000 | 51.1 | | |
| 11 July 2000 | 50.1 | | |

1. Linear calibration curve used for these samples had an $R^2$ value of 0.9992 (Appendix 1).

Table III-5. TCAA concentrations in two samples of Winnipeg water, one preserved and one not preserved, that were shipped subsequent to the data collection period for the exposure/intervention study[1].

| Unpreserved Water Stored in Plastic Container in Refrigerator | | Preserved Water Stored in Glass Bottle in Refrigerator | |
|---|---|---|---|
| **Date** | **TCAA Concentration (μg/L)** | **Date** | **TCAA Concentration (μg/L)** |
| January 29 2001 | 136.3 | January 29 2001 | 79.3 |
| January 30 2001 | 113.5 | January 30 2001 | 77.1 |
| January 31 2001 | 106.5 | January 31 2001 | 79.0 |
| February 1 2001 | 95.4 | February 1 2001 | 78.9 |
| February 2 2001 | 94.6 | February 2 2001 | 77.2 |
| February 9 2001 | 63.7 | February 9 2001 | 79.1 |

1. Linear calibration curve used for these samples had an $R^2$ value of 0.9993 using SPME-GC-ECD analysis (Appendix 2).

*TCAA Excretion*

The method used for TCAA analysis in urine allowed for sensitive detection of TCAA (MDL = 0.61 µg/L) and showed excellent repeatability (relative standard deviation of 5.6% on 7 triplicate analyses of urine). The amount of TCAA excreted (TCAA-exc) per day was calculated from the TCAA concentrations measured in the FMU samples, the volume of the samples, and the time interval between the previous urination and the FMU collection using the following equation:

$$\text{TCAA - exc}\left(\frac{\mu g}{d}\right) = \text{TCAA concentration in urine}\left(\frac{\mu g}{L}\right) \text{x Sample volume (L)} \\ \text{x Time interval}\left(\frac{1}{hr}\right) \text{x Day correction}\left(\frac{24\,hr}{d}\right) \quad (2)$$

A summary of the intra-individual variability of FMU TCAA excretion based on 12 days of tap water ingestion is outlined in Table III-6. These results show the relative standard deviation of FMU TCAA-exc ranged from a low of 27% to a high of 47% with an average TCAA-exc of 36%. In the previous pilot study, where ingestion sources and ingestion amounts were not managed, the intra-individual variability in TCAA excretion ranged from 15% to 71% (Froese et al. Submitted). The results of the current study indicate that when ingestion sources and amounts are controlled the range of TCAA excreted can be reduced. However, these results indicate that even when TCAA ingestion sources are highly managed there will be substantial intra-individual variability in TCAA excretion.

Table III-7 shows the inter-individual variability of FMU TCAA-exc calculated for all participants for 12 days of tap water ingestion ranges from a low of 31% to a high of 69% with an average TCAA-exc of 53%. Results of the Froese et al. (Submitted) study showed that the daily relative standard deviations for inter-individual variability of TCAA-exc ranged from 54% to 150%. Therefore, the results of this study indicate that when ingestion sources and volumes are controlled, the TCAA-exc variability between participants can be substantially reduced.

Table III-6. Intra-individual variability of TCAA excretion for all participants based on 12 days of tap water ingestion.

| Participant | Average Excretion (μg/d) | Standard Deviation | Relative Standard Deviation | Minimum Excretion (μg/d) | Maximum Excretion (μg/d) |
|---|---|---|---|---|---|
| WET-1[1] | 230 | 62 | 27% | 150 | 330 |
| WET-1[2] | 170 | 28 | 16% | 120 | 200 |
| WET-2[1] | 60 | 28 | 47% | 17 | 110 |
| WET-3[1] | 160 | 55 | 34% | 51 | 290 |
| WET-4[1] | 250 | 69 | 28% | 130 | 340 |
| WET-5[1] | 150 | 67 | 45% | 79 | 310 |
| Range[1] | 60 – 260 | 28 – 69 | 16% – 47% | 17 – 150 | 110 – 340 |

1. Based on FMU TCAA excretion
2. Based on all-day TCAA excretion

Table III-7. Inter-individual variability of TCAA excretion for all participants based on 12 days of tap water ingestion.

| Study Day | Average Excretion (µg/d) | Standard Deviation | Relative Standard Deviation | Minimum Excretion (µg/d) | Maximum Excretion (µg/d) |
|---|---|---|---|---|---|
| 2 | 170 | 110 | 65% | 50 | 280 |
| 3 | 160 | 110 | 69% | 46 | 330 |
| 4 | 130 | 59 | 45% | 73 | 230 |
| 5 | 200 | 120 | 60% | 49 | 340 |
| 6 | 150 | 47 | 31% | 89 | 190 |
| 7 | 200 | 120 | 60% | 17 | 340 |
| 8 | 160 | 75 | 47% | 95 | 280 |
| 9 | 150 | 62 | 41% | 64 | 230 |
| 10 | 200 | 100 | 50% | 54 | 340 |
| 11 | 180 | 110 | 61% | 19 | 310 |
| 12 | 170 | 89 | 52% | 79 | 290 |
| 13 | 170 | 85 | 50% | 58 | 260 |
| Range | 130 – 200 | 47 – 120 | 31% – 69% | 17 – 95 | 190 – 340 |

Because this study involved both male and female participants of varying ages, the intra-individual FMU TCAA-exc variability can be assessed according to both gender and age. The relative standard deviation range of TCAA-exc for the male participants was 27% to 34%, whereas the range for the female participants was 45% to 47%. This suggests that there may be greater intra-individual variability in urinary TCAA excretion for females than for males. These results are not likely influenced by the difference in ingestion volumes between the sexes, as ingestion volumes for males (relative standard deviation = 19%) did not vary substantially from females (relative standard deviation = 15%). Sex differences in urinary excretion of many compounds have been determined with hormonal regulation of the menstrual cycle in women being cited as, a reason for this difference (Singh et al. 1990). However, available literature is not specific to sex differences in urinary TCAA excretion and the limited data points from which the above correlation is made prevents confident judgement on whether there is a sex difference in urinary TCAA excretion.

Although the intra-individual variability for females was higher than that for males there was a smaller range of inter-individual variability between the female participants as compared to the male participants. A possible explanation for this finding is that the age range for males in this study (27 – 52 years) was much greater than the age range for the female participants (28 – 29 years). For all participants, there was an increasing intra-individual variability in TCAA-exc for decreasing age (Figure III-1) suggesting that the wider range in the intra-individual variability of TCAA-exc for the male participants was a function of the wider age range of the participants.

All-day urine collection by participant WET-1 enabled determination of how well TCAA-exc from FMU samples represents daily TCAA-exc. For the FMU samples daily TCAA-exc was estimated by adjusting the FMU excretion rate to 24 hr. Comparison of the TCAA-exc for participant WET-1 based on FMU TCAA excretion and all-day TCAA excretion shows that TCAA-exc from FMU samples (relative standard deviation 27%) is a close approximation of all-day TCAA excretion (relative standard deviation 16%) (Table III-6). This outcome increases confidence that FMU samples can be used as

representatives of daily TCAA excretion, thereby demonstrating the feasibility of using TCAA in FMU as a biomarker of exposure to DBPs.

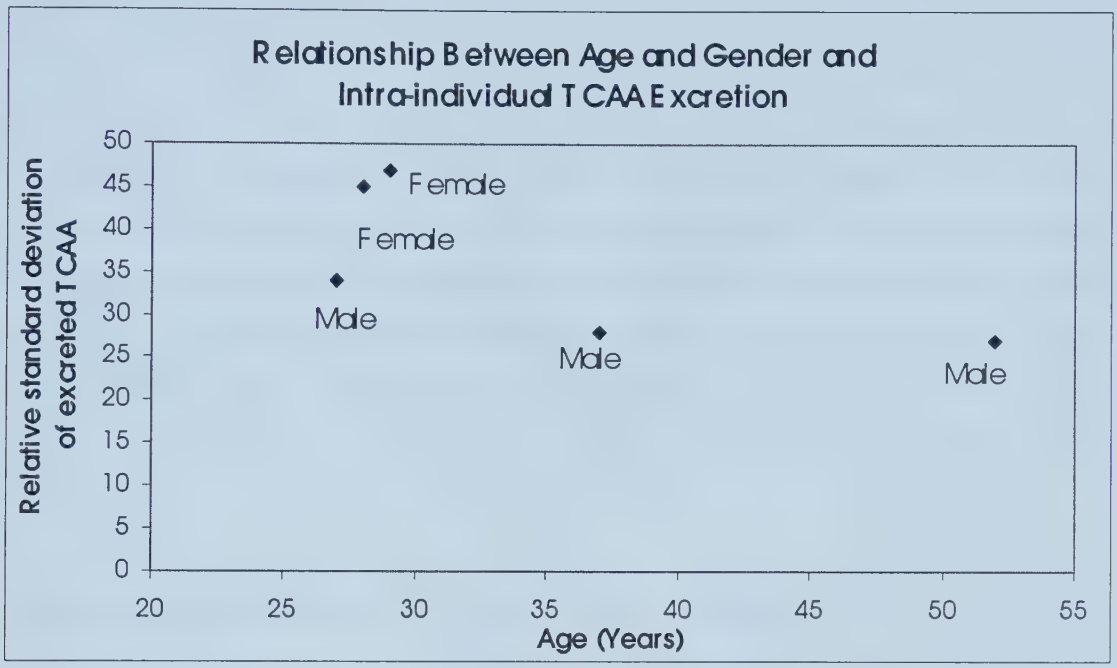


Figure III-1. Relationship between the age and gender of participants and relative standard deviation of TCAA excretion as an indicator of intra-individual variability.

Together, the TCAA ingestion and excretion results indicate intra- and inter-individual variability in both TCAA ingestion and excretion occurs despite management of TCAA ingestion sources and volumes. To date, there has been only one other study published that has evaluated using TCAA as a biomarker of exposure for DBPs (Kim et al. 1999; Weisel et al. 1999). This one study employed a cross-sectional design where urine and tap water samples were collected at the same time. Using this study design requires the assumption that water concentrations of TCAA remain constant for a sufficiently long time period before urine samples are collected so that daily variations in TCAA in drinking water do not affect urinary TCAA concentrations. Research shows that there is substantial daily variability in TCAA in chlorinated drinking water (Bodo 2001), therefore, the previous assumption does not accurately represent the actual conditions experienced in a water distribution system. Given the variability shown for TCAA ingestion and excretion, a cross-sectional design provides only a limited ability to assess

inter-individual TCAA ingestion and excretion and provides no information on intra-individual consumption and excretion of TCAA.

In addition to the above limitations, a cross-sectional design does not show the temporal trends for TCAA ingestion and excretion and does not correct TCAA ingestion for the contribution of previous days ingestion. Given that intra-individual variability was shown previously to range from 43% to 52% for TCAA-ing and from 27% to 47% for TCAA-exc when ingestion sources and volumes were highly managed, not accounting for temporal variations in TCAA ingestion and excretion or correcting TCAA-exc for previous day's ingestion introduces uncertainty into the ingestion-excretion estimate. The longitudinal design of this study allowed for temporal visualization of TCAA-ing and TCAA-exc and for correction of previous day's TCAA ingestion on urinary TCAA excretion.

*Temporal Relationship between TCAA Ingestion and Excretion*

To assess the temporal relationship of TCAA ingestion and excretion, excreted TCAA was back-adjusted one day to correspond to the previous 24 hr of TCAA ingestion. Figure III-2 shows the relationship between TCAA-ing and TCAA-exc averaged for all participants for each day of the study. Ingestion and excretion are closely matched for study days 4 through 9, which is possibly a function of the higher TCAA-exc than TCAA-ing for two individual participants. The sharp increase in TCAA-ing on study day 10 for the average of all participants reflects the higher TCAA concentration in the second shipment of Winnipeg tap water as compared to the first shipment and large ingestion volumes for participants WET-1 and WET-4 in response to high ambient temperature and physical exercise.

Figures III-3 and III-4 illustrate the relationship between TCAA-ing and TCAA-exc for participant WET-1 based on FMU and all-day urine, respectively. These two figures show that the relationship between ingested TCAA and TCAA excreted in FMU is a good approximation of the temporal relationship between ingested TCAA and the TCAA excreted in all-day urine. Figure III-3 also shows TCAA-exc was higher than TCAA-ing

for days 3 to 10 for this participant based on FMU excretion, therefore, contributing to the close relationship between TCAA-exc and TCAA-ing shown for the average of all participants on study days 4 to 9. The closer relationship between TCAA-ing and

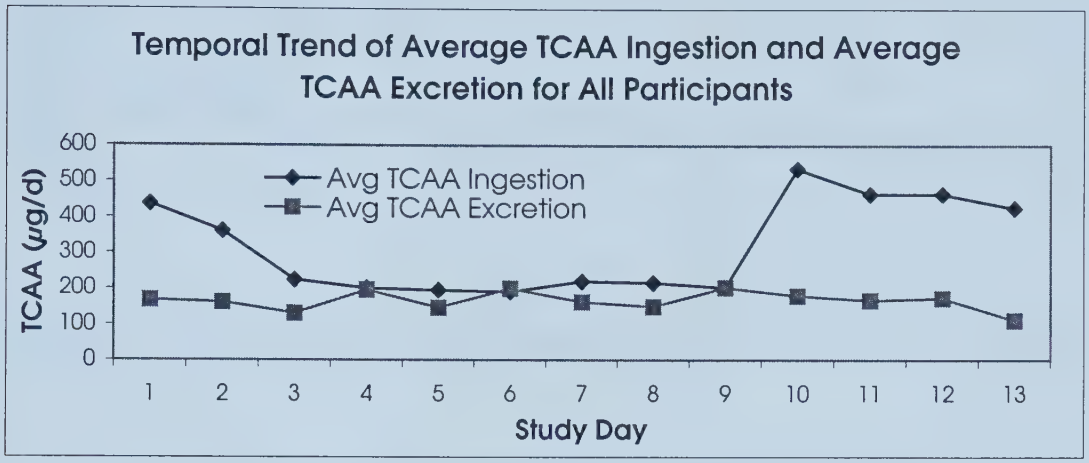


Figure III-2. Temporal trend of TCAA-ing and TCAA-exc averaged for all participants for 13 days of bottled water ingestion.

TCAA-exc shown in for participant WET-1 based on all-day excreted TCAA (Figure III-4) is explained by the contribution of FMU to overall daily TCAA excretion. On average, the proportion of all-day TCAA that was excreted in FMU was 38% (± 26% relative standard deviation). This proportion of TCAA-exc over a smaller time frame leads to the higher values for TCAA-exc calculated from FMU as compared to all-day excretion.

The temporal relationships between TCAA-ing and TCAA-exc for participants WET-2 to WET-5 are shown in Figures III-5 to III-8. The high TCAA-ing value for participant WET-2 on study day 10 (Figure III-5) is a function of the high TCAA concentration in the second shipment of Winnipeg water on that day and an increased consumption volume relative to the other study days. Figures III-6 and III-8 show a consistent trend between TCAA-ing and TCAA-exc for participants WET-3 and WET-5 for the days of tap water exposure.

Analysis of Figure III-7 shows that participant WET-4, like participant WET-1, had a higher TCAA-exc than TCAA-ing for days 4, 6, 7, 8, and 9 of the tap water ingestion exposure. Review of the daily diaries for participant WET-4 showed this participant

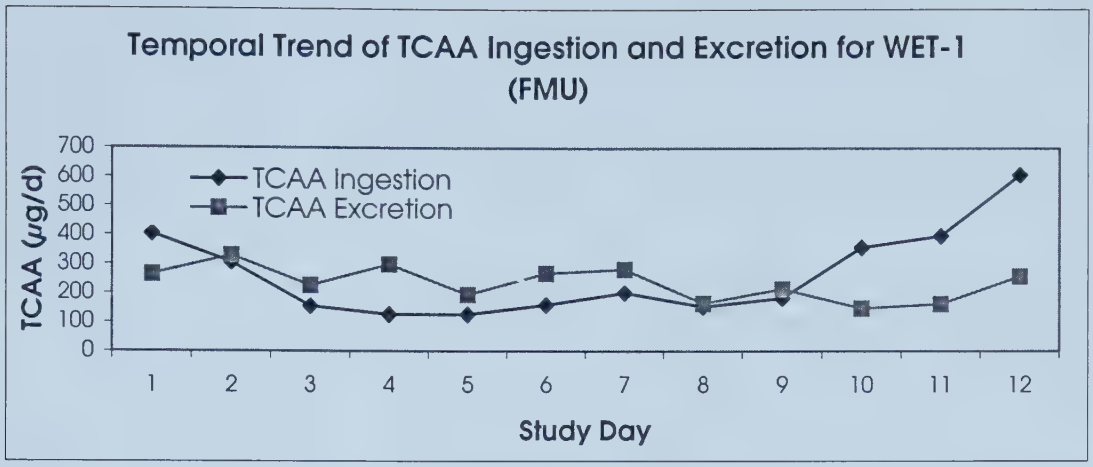


Figure III-3. Temporal trend of TCAA-ing and TCAA-exc for WET-1 using all-day TCAA excretion.

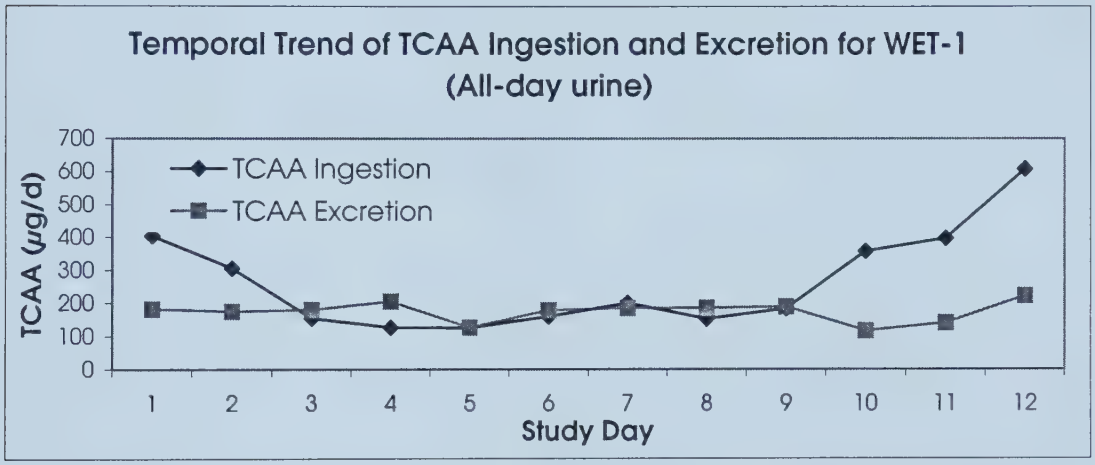


Figure III-4. Temporal trend of TCAA-ing and TCAA-exc for WET-1 using FMU TCAA excretion.

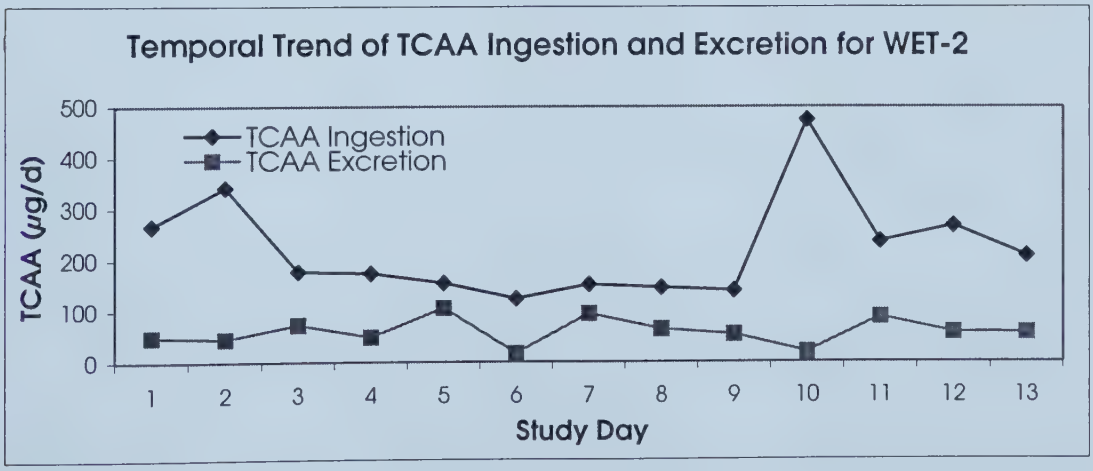


Figure III-5. Temporal trend of TCAA-ing and TCAA-exc for WET-2 using FMU TCAA excretion.

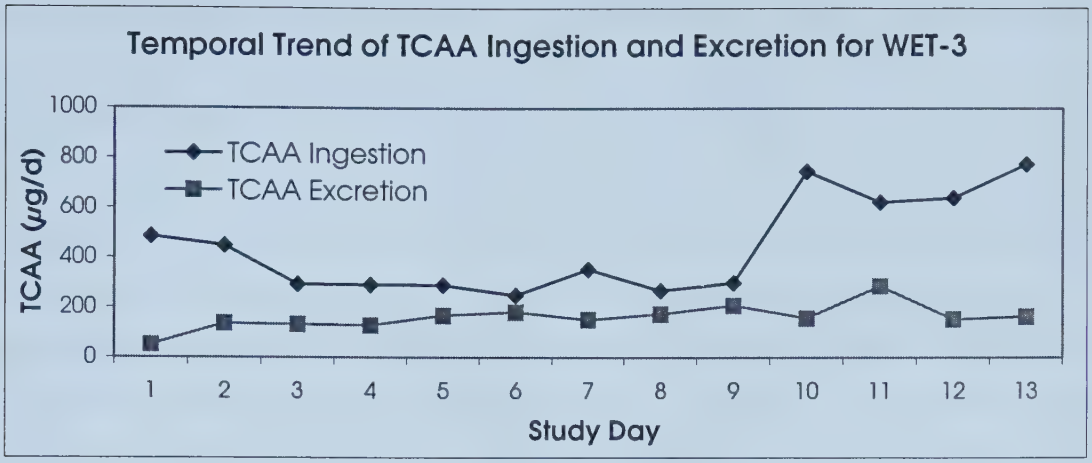


Figure III-6. Temporal trend of TCAA-ing and TCAA-exc for WET-3 using FMU TCAA excretion.

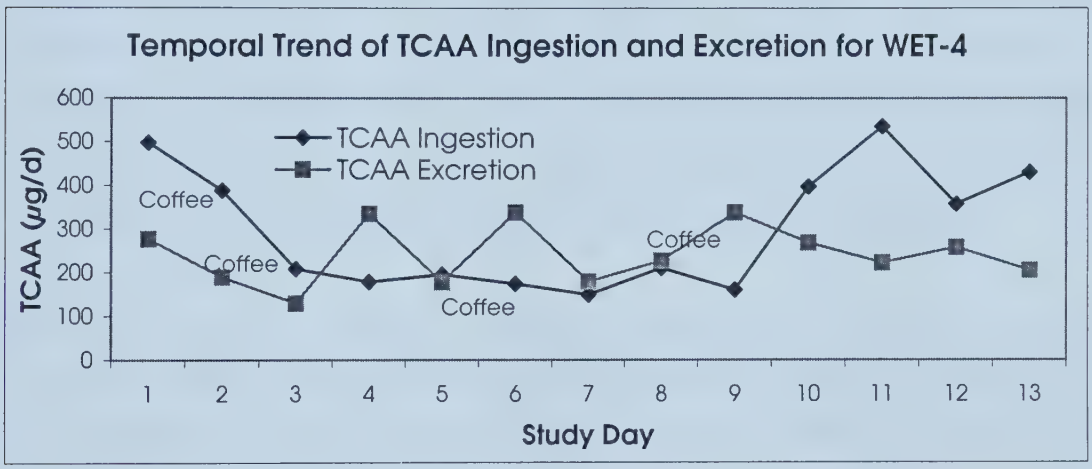


Figure III-7. Temporal trend of TCAA-ing and TCAA-exc for WET-4 using FMU TCAA excretion.

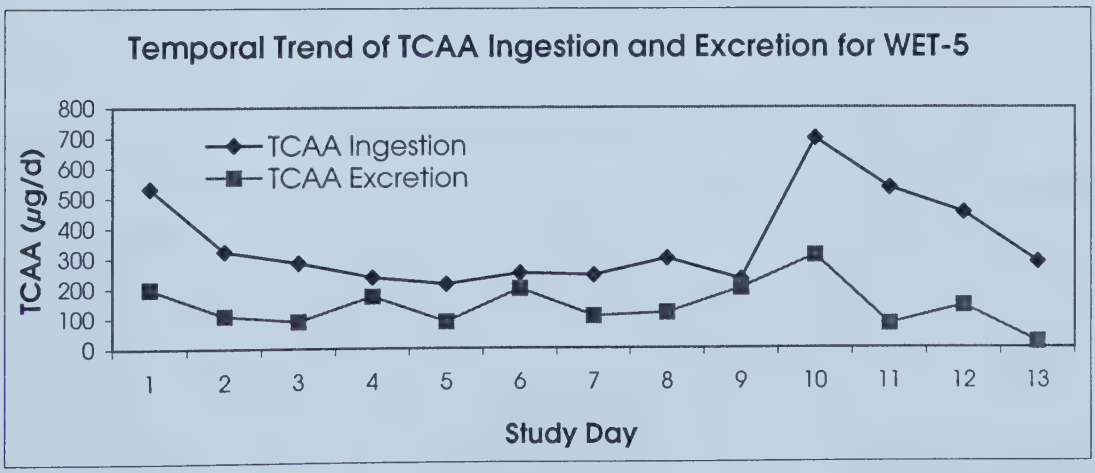


Figure III-8. Temporal trend of TCAA-ing and TCAA-exc for WET-5 using FMU TCAA excretion.

recorded consuming coffee on days 2, 3, 6, and 8 of the tap water study days. Coffee made with chlorinated tap water has approximately double the levels of TCAA over chlorinated tap water itself due to the interaction of free chlorine with additional organic matter introduced in the form of ground coffee (Balko et al. 2001). The extent coffee consumption contributed to excreted TCAA was investigated by a worst-case scenario approximation. Participant WET-4 indicated in the daily diaries that any coffee consumed was prepared with Edmonton tap water. Levels of TCAA in Edmonton tap water did not exceed 10 μg/L for the duration of the study based on routine monitoring. Therefore, the expected maximum concentration of TCAA in the coffee consumed by participant WET-1 used for the calculation was 20 μg/L (based on a relative doubling of TCAA when coffee is prepared with chlorinated water). Using this TCAA concentration in coffee and the volumes of coffee consumed the amount of TCAA contributed by coffee per day was calculated to be 3, 3, 3, and 2 μg for days 2, 3, 6, and 8, respectively. Therefore, it is not likely that coffee consumption by participant WET-4 contributed substantially to the higher TCAA-exc than TCAA-ing for this participant.

The exposure/intervention design of this study allowed for the measurement of individual TCAA elimination half-lives for all of the participants and showed that participant WET-1 eliminated TCAA more slowly than the other participants. Half-life values for the five participants, as determined from FMU creatinine-normalized TCAA excretion versus time curves, were 5.0, 2.1, 6.3, 2.3, and 2.5 for participants WET-1, 2, 3, 4, and 5, respectively (Chapter II). This shows that participant WET-1 had a TCAA elimination half-life twice as long as the other participants, with the exception of participant WET-3. Also, the longer half-life determined for participant WET-3 is possibly a function of this participant being exposed to TCAA from regular swimming. The half-life determined for participant WET-1 suggests that this participant eliminated TCAA at a slower rate than the other participants (except WET-3), raising the possibility that the higher TCAA-exc than TCAA-ing for participant WET-1 was a function of slow elimination of ingested TCAA that occurred prior to the onset of the study.

*Correlation between TCAA Ingestion and Excretion*

Determining the capability of using urinary TCAA as a biomarker of exposure to DBPs requires a confident relationship between TCAA ingestion and TCAA excretion. The longitudinal design of this study allowed for determination of the correlation between TCAA ingestion and excretion both intra-individually and inter-individually according to various corrections for matching TCAA ingestion and excretion.

The average ratio of TCAA-exc to TCAA-ing based on FMU TCAA excretion for each participant showing the intra-individual variability for all days of tap water consumption is presented in Table III-8. For this comparison, TCAA-exc was back-adjusted one day to correspond to the previous 24 hr of TCAA ingestion. The range of relative standard deviations (50% – 60%) shows a reasonably consistent variation in the average ratio of TCAA-exc to TCAA-ing for all participants. This consistent ratio is likely influenced by the consistent range of intra-individual ingestion variability (43% – 52%, Table III-1) dictated by the control over TCAA ingestion sources and volumes.

Observation of the average excretion-ingestion ratios for all participants (Table III-8) shows the excretion-ingestion proportions determined for participants WET-1 (1.1) and WET-4 (1.0) were substantially different than the proportions determined for WET-2 (0.32), WET-3 (0.43), and WET-5 (0.43). The average of the excretion-ingestion proportions determined for participants WET-2, 3, and 5 (0.39) is consistent with literature values for the recovery of administered TCAA in urine (0.49) except that these were at much larger doses (Humbert et al. 1994), (0.47) (Muller et al. 1974). The higher excretion-ingestion ratios determined for WET-1 and WET-4 are likely related to the increased age of participants WET-1 and WET-4 relative to the other participants. The average age of participants WET-1 and WET-4 was 45 years whereas the average age of participants WET-2, WET-3, and WET-5 was 28 years, suggesting that the older participants excreted a larger proportion of the ingested TCAA than the younger participants (Figure III-9). Figure III-9 also shows that the higher proportion of TCAA-exc to TCAA-ing for participants WET-1 and WET-4 is more likely a function of age and not related to gender.

Table III-8. Proportion of TCAA excreted from previous day's TCAA ingestion showing intra-individual variability.

| Participant | Average | Standard Deviation | Relative Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| WET-1[1] | 1.1 | 0.60 | 55% | 0.41 | 2.4 |
| WET-1[2] | 0.85 | 0.42 | 49% | 0.32 | 1.6 |
| WET-2[1] | 0.32 | 0.19 | 59% | 0.040 | 0.69 |
| WET-3[1] | 0.43 | 0.20 | 50% | 0.10 | 0.73 |
| WET-4[1] | 1.0 | 0.60 | 60% | 0.42 | 1.9 |
| WET-5[1] | 0.43 | 0.24 | 56% | 0.065 | 0.87 |
| Range[1] | 0.32 – 1.1 | 0.19 – 0.60 | 50% – 60% | 0.040 – 0.42 | 2.4 |

1. Based on FMU TCAA excretion.
2. Based on all-day TCAA excretion.

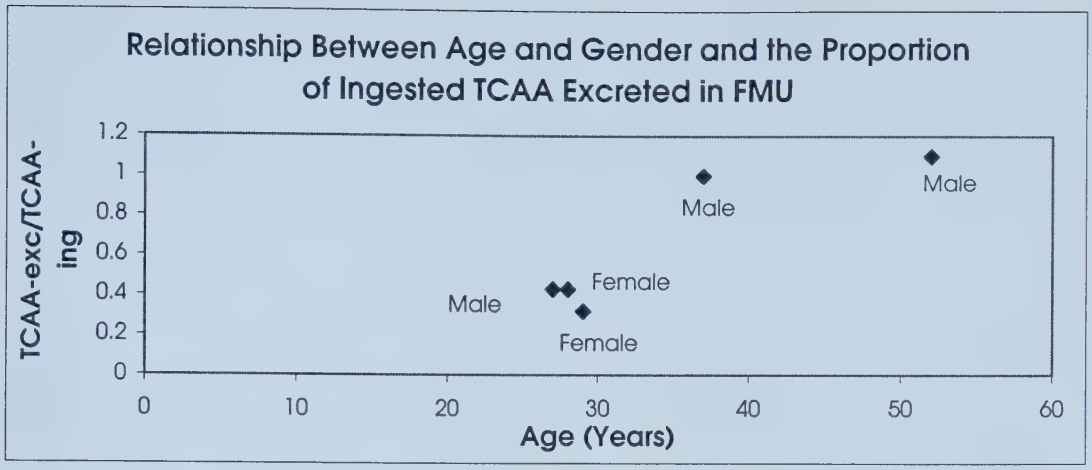


Figure III-9. Relationship between the proportion of ingested TCAA that is excreted and age and gender.

Table III-9 shows the inter-individual variability in the proportion of TCAA excreted from the previous day's TCAA ingestion. These results show the inter-individual variability of the ratio of excreted to ingested TCAA ranges from 51% – 87%. The higher relative standard deviations for days 4 to 10 (71% – 87%) reflect the higher TCAA ingestion than excretion for participants WET-1 and WET-4.

Linear regression analysis was performed to evaluate the strength of the correlation between TCAA-ing and TCAA-exc (for both the one-day back adjusted excretion and average of three previous day's ingestion) using daily TCAA-ing as the independent variable and daily TCAA-exc as the dependent variable for the 12 d tap water ingestion period. This correlation was performed using the assumption that daily TCAA-exc extrapolated from a single FMU sample is linearly related to the average TCAA-ing from the previous 24 hours or three days. The small range of TCAA ingestion variability between participants (16% – 58% (average 33%), Table III-2) predetermined by the study design resulted in the relatively small range of inter-individual variability in TCAA excretion (31% – 61%, Table III-7). This prevented demonstrating a relationship between TCAA-ing and TCAA-exc based on the one-day back-adjusted TCAA excretion or average of three previous day's ingestion. Further, this finding demonstrates that ingestion exposures must differ by greater than 30% in order to determine an exposure-excretion relationship for TCAA in urine.

Table III-9. Proportion of TCAA excreted from previous day's TCAA ingestion showing inter-individual variability.

| Study Day | Average | Standard Deviation | Relative Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| 1 | 0.35 | 0.20 | 57% | 0.10 | 0.56 |
| 2 | 0.38 | 0.20 | 51% | 0.13 | 0.65 |
| 3 | 0.58 | 0.30 | 52% | 0.32 | 1.1 |
| 4 | 0.96 | 0.68 | 71% | 0.29 | 1.9 |
| 5 | 0.99 | 0.79 | 79% | 0.42 | 2.4 |
| 6 | 1.0 | 0.71 | 69% | 0.13 | 1.9 |
| 7 | 0.88 | 0.55 | 63% | 0.44 | 1.7 |
| 8 | 0.79 | 0.43 | 55% | 0.39 | 1.4 |
| 9 | 1.0 | 0.65 | 64% | 0.38 | 2.1 |
| 10 | 0.51 | 0.44 | 87% | 0.040 | 1.2 |
| 11 | 0.36 | 0.12 | 34% | 0.15 | 0.42 |
| 12 | 0.38 | 0.21 | 54% | 0.31 | 0.72 |
| 13 | 0.29 | 0.17 | 57% | 0.065 | 0.48 |
| Range | 0.29 – 1.0 | 0.12 – 0.79 | 34% – 87% | 0.040 – 0.44 | 0.48 – 2.4 |

Because this study involved an intervention period where participants consumed TCAA-free bottled water, determination of an ingestion-excretion correlation for a wider range of TCAA-ing and TCAA-exc values was possible. In addition, the measurement of elimination rate constant values for each of the participants in this study allowed determination of the correlation between TCAA ingestion and excretion using the expected TCAA excretion adjusted for the previous days' ingestion [Ex(e)] according to exponential decline and the measured TCAA excretion [Ex(m)]. This accounts for the contribution of prior TCAA ingestion carrying over to measured TCAA excretion. The elimination rate constant adjusted TCAA elimination [Ex(e)] was calculated using a running average of ingestion that accounted for the TCAA ingested from previous days according to exponential elimination using equation 3. After two half-lives from a given day less than 25% of excretion on the later day remains from the TCAA ingested on the given earlier day. Based on this small contribution equation 3 was carried through two half-lives for each participant using the respective half-lives determined for each participant. One third of a day was chosen for the day fraction based on the estimation that TCAA excreted in FMU reflects TCAA that has collected over eight hours. For simplicity, equation 3 assumes 100% absorption of ingested TCAA across the gastrointestinal tract and 100% excretion of ingested TCAA.

$$\mathrm{Ex(e)}\left(\frac{\mu g}{d}\right) = \frac{\Sigma\left[\text{Previous day's ingestion}(\mu g) \times \exp - \left(\text{Day fraction(d)} \times k_{el}\right)\right]}{\text{number of days}} \qquad (3)$$

The correlations between measured TCAA excretion and half-life adjusted TCAA expected excretion for participants WET-1 to WET-5 are shown in Figures III-10 to III-14. These results show a reasonable correlation between TCAA ingestion and excretion over a wide range of ingestion and excretion values providing R values of 0.64 (n=23, WET-2), 0.70 (n=15, WET-3), 0.72 (n=22, Wet-5), 0.80 (n=22, WET-4), 0.92 (n=25, WET-1) which all are significant at $P<0.005$. It is possible that the R values for these correlations are influenced by the zero values produced for expected excretion, dictated by the equation used for their calculation. Without the zero values for Ex(e) the R values for participants WET-1 to WET-5 are 0.85 (n=14), 0.32 (n=14), 0.66 (n=14), 0.57 (n=14),

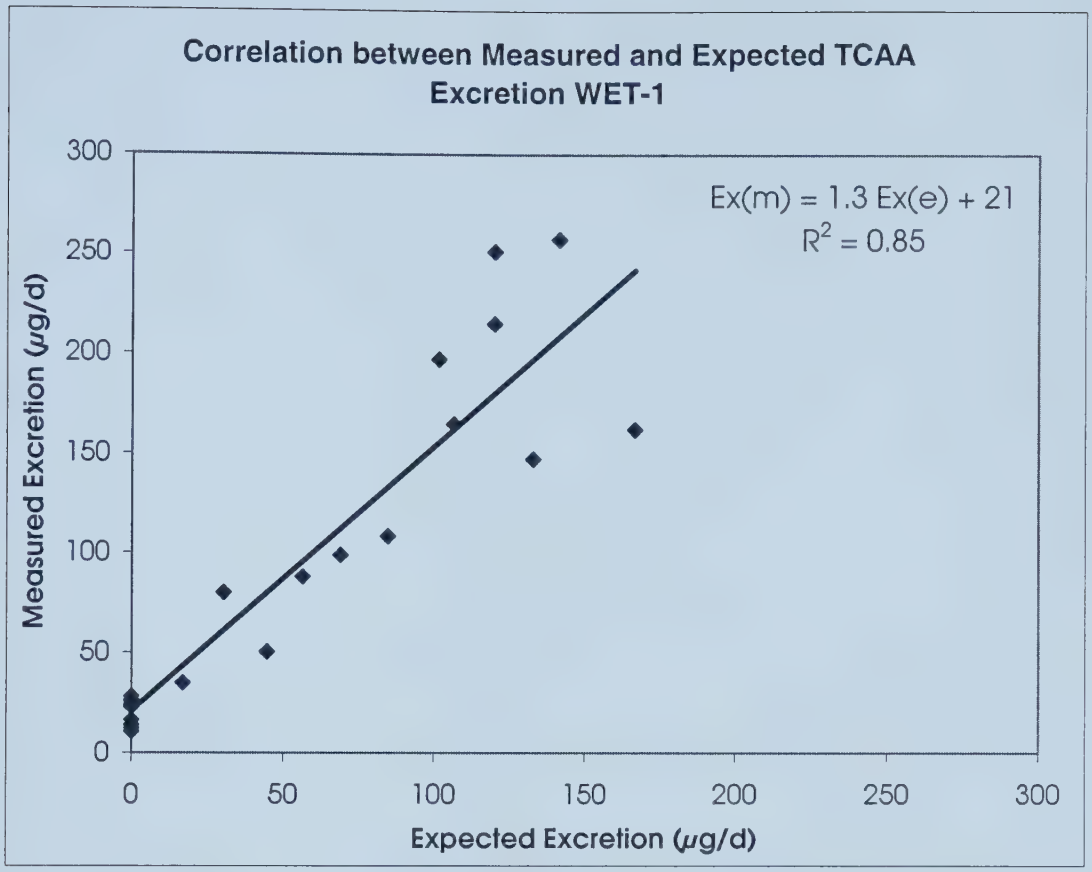


Figure III-10. Correlation of measured TCAA excretion [Ex(m)] and half-life adjusted expected TCAA excretion [Ex(e)] for WET-1 (R=0.92, P<0.005, n=25)

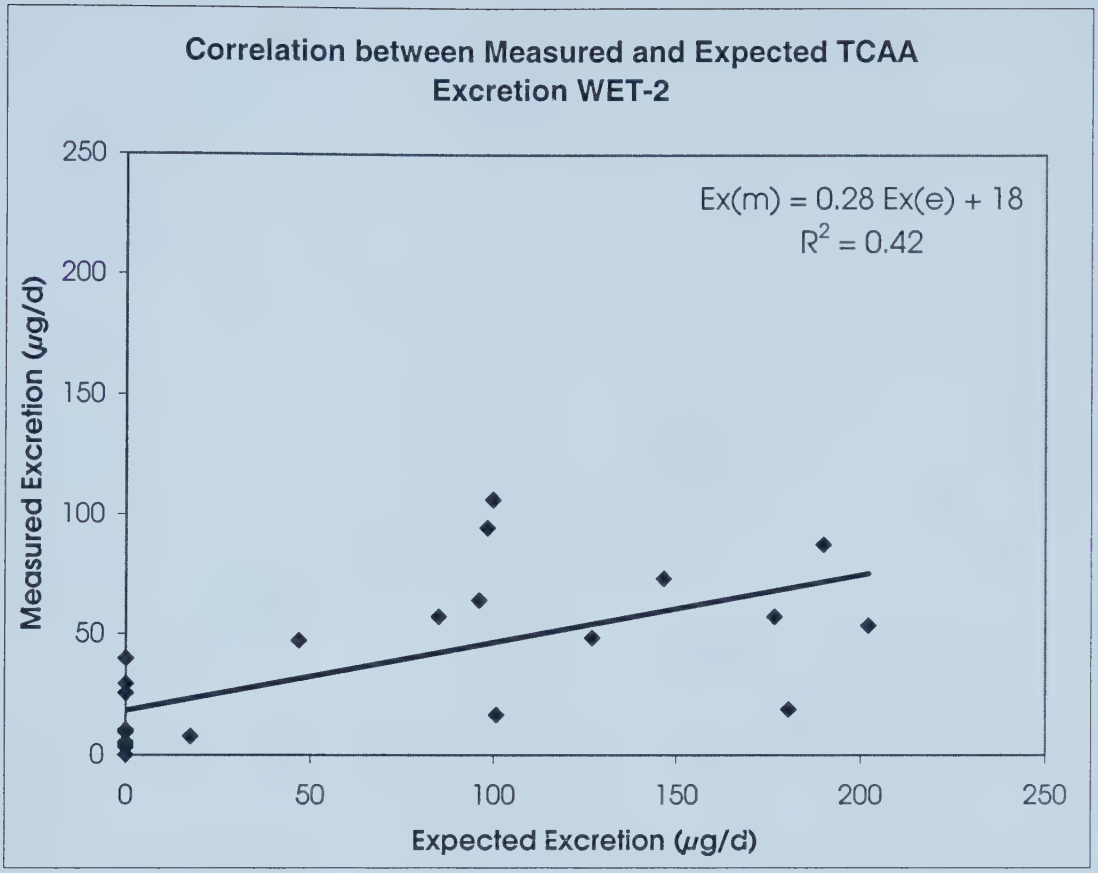


Figure III-11. Correlation of measured TCAA excretion [Ex(m)] and half-life adjusted expected TCAA excretion [Ex(e)] for WET-2 (R=0.64, P<0.005, n=23)

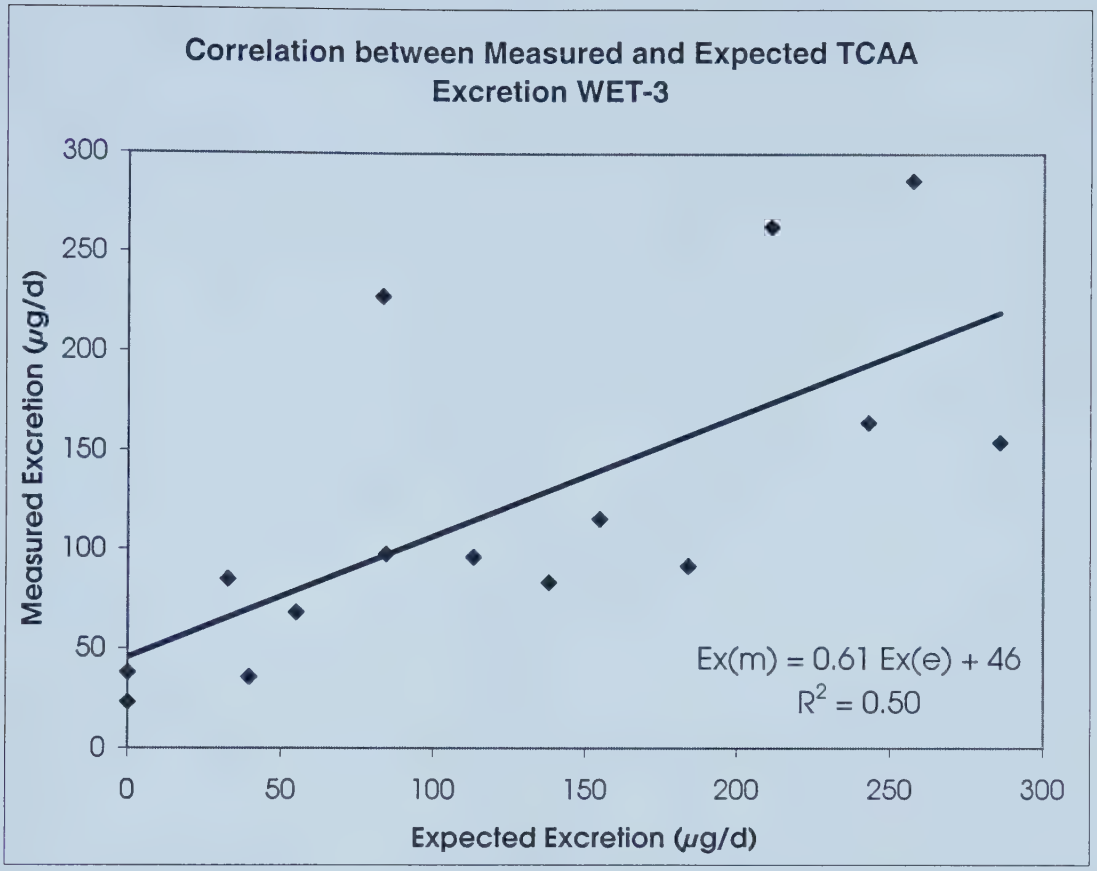


Figure III-12. Correlation of measured TCAA excretion [Ex(m)] and half-life adjusted expected TCAA excretion [Ex(e)] for WET-3 (R=0.70, P<0.005, n=15)

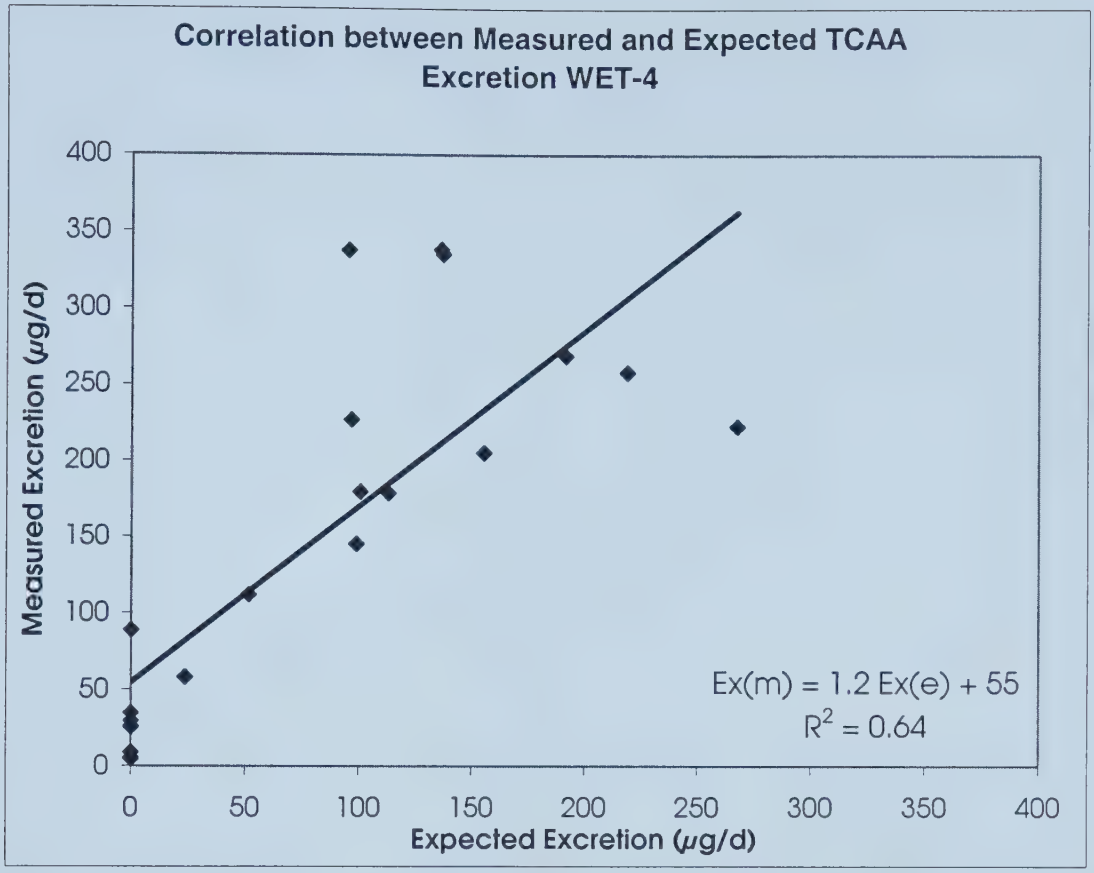


Figure III-13. Correlation of measured TCAA excretion [Ex(m)] and half-life adjusted expected TCAA excretion [Ex(e)] for WET-4 ($R=0.80$, $P<0.005$, $n=22$)

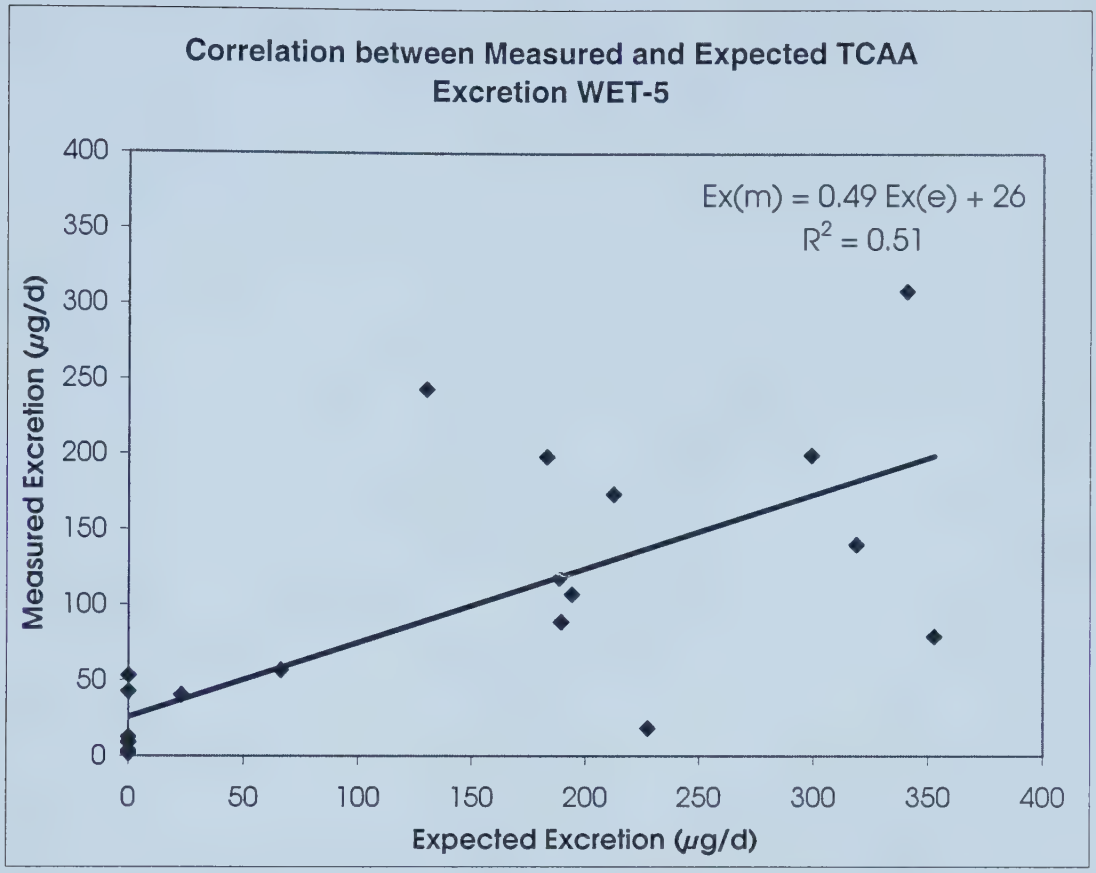


Figure III-14. Correlation of measured TCAA excretion [Ex(m)] and half-life adjusted expected TCAA excretion [Ex(e)] for WET-5 (R=0.72, P<0.005, n=22)

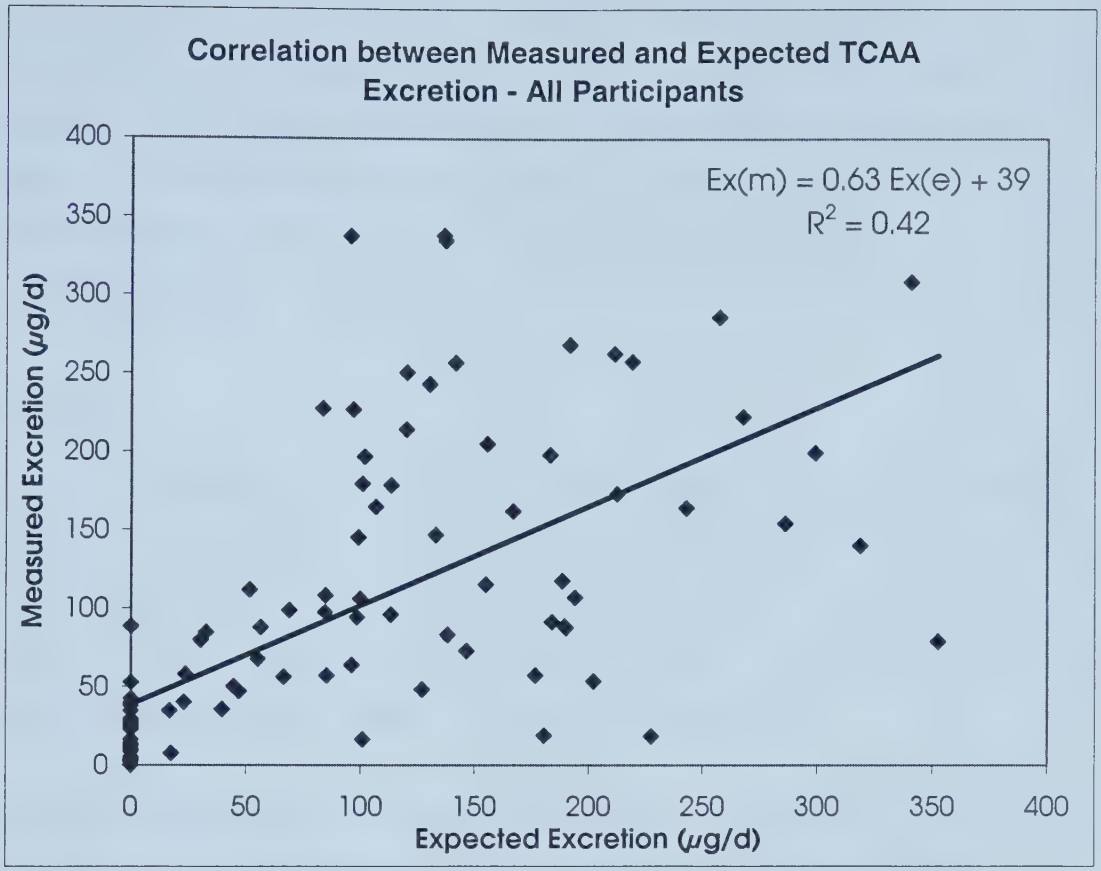


Figure III-15. Correlation of measured TCAA excretion [Ex(m)] and half-life adjusted expected TCAA excretion [Ex(e)] for all participants for all days of the study (R=0.65, P<0.005, n=22).

and 0.47 (n=14), respectively. This possibility warrants further investigation. The correlation between measured and half-life adjusted expected TCAA excretion for all participants for all of the study days is shown in Figure III-15 ($R=0.65$, $P<0.005$ for $n=105$). In total, these correlations are consistent with results provided by Froese et al. (Submitted) from a similar longitudinal exposure-intervention study ($R=0.74$, 0.84, $P<0.005$ for $n=10$).

### *Conclusions*

Improved confidence in using TCAA as a valid biomarker for exposure to DBPs is provided by the results of this study. Variability in individual consumption patterns was shown to influence TCAA ingestion and excretion variability, despite substantial control over ingestion sources and volumes. This demonstrates the value of prospective water consumption records to accurately document TCAA exposures.

Increased confidence in the feasibility of using FMU as a representative of total daily urinary excretion was also shown. The potential for TCAA in FMU samples to overestimate daily urinary excretion was indicated and further investigation of this outcome is required to maximize confidence in using TCAA excreted in FMU samples.

Higher TCAA ingestion exposures correlated with higher urinary TCAA excretion based on a wide range of data points using expected TCAA excretion adjusted for half-life. The failure of ingested TCAA to correlate with excreted TCAA without the additional data points from the intervention experiment by using half-life to account for excretion contribution from previous days indicates that individual TCAA ingestion exposures need to differ by greater than about 30% for biomarker studies to demonstrate a correlation between excretion and ingestion.

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# IV General Discussion and Conclusion

Findings from toxicologic studies performed by exposing animals to high levels of individual disinfection by-products (DBPs) suggest that some by-products of disinfection cause reproductive failures (EPA/ILSI 1993). Although there have been epidemiologic studies conducted to investigate if there is a similar relationship between exposure to DBPs at drinking water levels and the occurrence of adverse reproductive outcomes in humans, these studies have been limited in their ability to assess causality because of inaccurate ascertainment of individual exposure (Bove et al. 1995; Savitz et al. 1995; Gallagher et al. 1998; Swan et al. 1998; Waller et al. 1998; Dodds et al. 1999; Magnus et al. 1999; King et al. 2000; Yang et al. 2000). Traditionally, exposure status for participants in epidemiologic studies has been assessed according to a combination of water source, chlorination practice, levels of DBPs routinely measured in municipal water, and personal water use questionnaires. The many different ways and amounts in which people use water and the respective physiochemical properties of individual DBPs complicate assessing exposure to DBPs in drinking water, making these general exposure assessment methods inaccurate for assessing individual exposure to DBPs.

Biomarkers of exposure are currently being investigated as replacements and/or supplements to the traditional methods of determining exposure in epidemiologic studies because they provide individual-level exposure assessment. Using exposure biomarkers to improve studies of the relationship between DBPs and adverse reproductive effects, however, requires them to be available, validated, and feasible. Trichloroacetic acid (TCAA), one individual DBP, has recently shown promise as an available exposure biomarker for DBPs when measured in urine (Kim et al. 1999; Weisel et al. 1999). The applicability of TCAA as an exposure biomarker for DBPs was investigated in this thesis and the results can be considered according to the desirable characteristics for biomarkers (Chapter I).

Using TCAA as a biomarker requires that it is persistent enough in the human body to encompass successive exposure intervals to TCAA from drinking water. In Chapter II, elimination half-life of TCAA in urine was determined using human participants: the

elimination half-life for TCAA ranges from 2.1 to 6.3 d for this study. This shows that TCAA has an elimination half-life that is significantly greater than the time interval between successive exposures to TCAA from intermittent daily consumption. Because the elimination half-life is several times the interval between repeated exposures, TCAA will accumulate in plasma towards a quasi-steady state level. Assuming that urine concentrations parallel plasma concentrations, the urinary excretion of TCAA would reflect average exposure or dose over several days. Therefore, TCAA in urine appears to be persistent enough in the human body to provide a valid exposure biomarker for ingested TCAA from chlorinated drinking water.

Determination of the elimination half-life of TCAA when TCAA is administered at drinking water levels was first examined by Froese et al. (Submitted) in a longitudinal pilot study investigating the validity of TCAA as an exposure biomarker (half-life range 2.3-3.7 d). Prior to that study, the only literature available for the elimination half-life of TCAA in humans was from TCAA as a metabolite of chloral hydrate (CH) (half-life range 2.9-5.0 d) (Breimer et al. 1974; Humbert et al. 1994) or trichloroethylene (TCE) (half-life 4.2 d) (Bruning et al. 1998) or from TCAA administered at doses that are substantially higher equivalent concentration levels than those that occur in drinking water (half life range 2.1-4.1 d) (Paykoc and Powell 1945; Muller et al. 1972; Muller et al. 1974). The results from this study confirm that the reported literature values for TCAA, as a metabolite of other compounds or as a parent compound administered at high doses, accurately represent the elimination half-life of TCAA when TCAA is given at drinking water concentrations.

There is improved confidence in the half-life values for TCAA in drinking water determined in this study as compared to the Froese et al. (Submitted) study. The half-life values determined in this study have corresponding coefficients of determination ($R^2$) of 0.76-0.94, whereas the coefficients of determination in the former study range from 0.45 to 0.74 (Froese et al. Submitted). An important distinction between the Froese et al. study and this study is that for this study the $R^2$ values were determined from a greater number of data points. This prevents directly comparing the respective $R^2$ values.

However, given that for a greater number of data points the $R^2$ values are substantially higher, we can be more confident that the half-life values in this study represent the true human elimination half-life values for TCAA in urine when TCAA is administered at drinking water levels.

The relationship between ingested and excreted TCAA shows that higher TCAA ingestion exposures correlate with higher urinary excretion levels (Chapter III). This finding fulfills a primary prerequisite for applying biomarkers in epidemiologic studies – that changes in biomarker TCAA levels reflect changes in TCAA exposure levels – and supports the findings of Kim et al. (1999), Weisel et al. (1999), and Froese et al. (Submitted) that TCAA appears to be a valid biomarker of exposure to DBPs when measured in urine. Intra- and inter-individual variability in TCAA ingestion and excretion were assessed and the results show that these variables can be reduced when TCAA ingestion sources and volumes are controlled. However, individual differences in consumption patterns and elimination kinetics continue to influence these variables despite substantial management of ingestion.

The findings presented here consistently show increased confidence in the feasibility of using TCAA in urine as a valid exposure biomarker for DBPs. Urinary sampling of TCAA using first morning urine (FMU) samples is relatively non-invasive and easy to collect. Because TCAA in FMU appears to be representative of daily TCAA excretion (Chapter II), increased confidence in the feasibility of biomarker use can possibly be achieved by using FMU samples. The finding that FMU samples can over-estimate the amount of TCAA excreted per day (Chapter III) requires further investigation. The results of this thesis also show that TCAA is easily detectable in the urine of people exposed to TCAA in chlorinated tap water using the methods described and that urinary TCAA levels are low after TCAA-ingestion sources are eliminated. TCAA in urine was stable over the time required to complete the analyses for this study (Chapter II) and subsequent analyses indicated that TCAA in urine is stable for up to two weeks (Chapter II), increasing confidence in the feasibility of using biomarker levels of TCAA in urine in epidemiologic studies.

The quality of the results of this thesis was likely achieved by the high level of control exerted over TCAA ingestion sources and water volumes (Chapter II). Future studies examining the validity and feasibility of TCAA as an exposure biomarker for DBPs when TCAA ingestion is not as highly controlled would strengthen our understanding of the applicability of using TCAA as a DBP exposure biomarker. The results of this thesis also indicate other important requirements for future DBP exposure biomarker investigation. One important requirement that was shown in this thesis is prospective ascertainment of water consumption and other water-related activities, because ingestion volume was shown to substantially influence TCAA ingestion, and other water-related activities appeared to influence TCAA exposure for certain participants. Without using the additional values for TCAA ingestion afforded by calculating expected excretion using elimination half-life, there was no correlation between the TCAA ingestion exposures and excretion levels dictated by the study design. This latter relationship suggests that ingestion exposures need to vary over a range of more than 30% to obtain a meaningful relationship between ingested and excreted TCAA. Future study designs should dictate that TCAA concentrations between exposure groups (or exposed individuals) differ by greater than 30% to ensure differences in TCAA excretion are observed. Also, it is recommended that the water used in these exposure studies have stable levels of TCAA to eliminate the possibility of TCAA fluctuation influencing the results and that the fate of TCAA in unpreserved water that is stored in plastic containers be investigated.

It is only recently that the relationship between biomarker levels of TCAA have been examined and the methods currently used are not practical for implementation into large-scale epidemiologic studies. The findings presented here lend support to using TCAA as a biomarker of exposure to DBPs in epidemiologic investigation. Results of any future epidemiologic studies using exposure biomarkers for exposure assessment will provide important insight to the regulation of DBPs in public water supplies. Current regulation of DBPs in drinking water limits total trihalomethanes to 100 μg/L and these regulations are subject to continual review (Canada 1996). Regulating DBPs in drinking water requires a careful balance of reducing exposure to DBPs while maintaining control of waterborne disease.

Any effort to reduce the potential health risks associated with DBPs must not compromise the biological water quality currently obtained by chlorination. The occurrence of seven fatalities in Walkerton, Ontario in May 2000 because of inadequate disinfection highlights how critical is this element of drinking water treatment. However, while the known health risks from microbial contaminants in drinking water are substantial as compared with the theoretical risks from chemical by-products, there remains uncertainty as to the actual risks posed by chlorination by-products. If the positive findings suggested by the current epidemiologic and toxicologic research on the relationship between exposure to DBPs and adverse reproductive outcomes are confirmed, the presence of DBPs in drinking water constitutes a substantial public health problem. Although the relative risks are low, a large part of the population is exposed to DBPs at some level due to the ubiquitous presence of DBPs in chlorinated drinking water. This translates into a potentially significant absolute risk. Thus, there is a need for the work presented in this thesis to improve exposure assessment in the epidemiologic method and, therefore, advance understanding of the relationship between exposure to DBPs and adverse reproductive outcomes.

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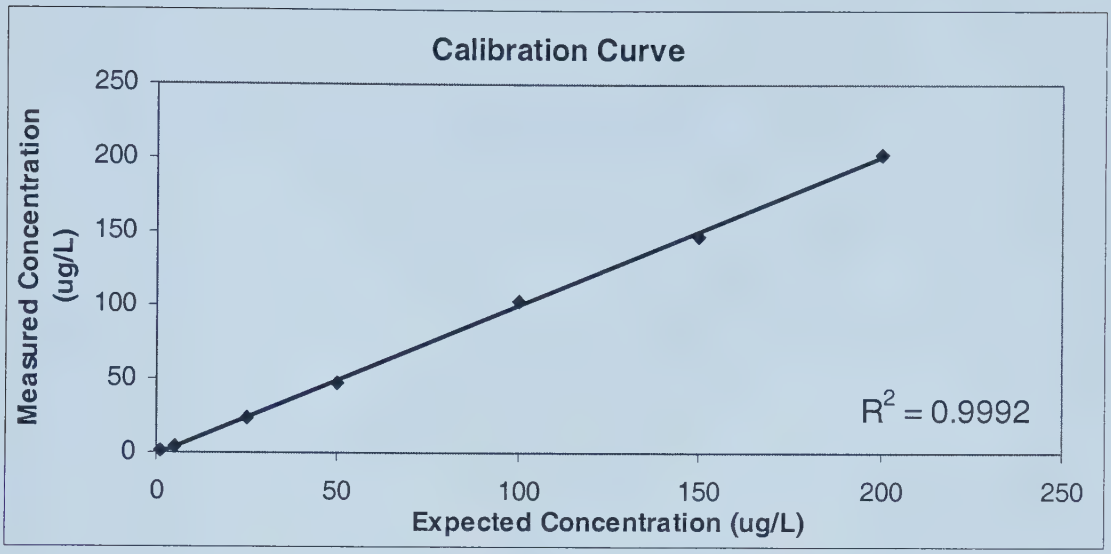
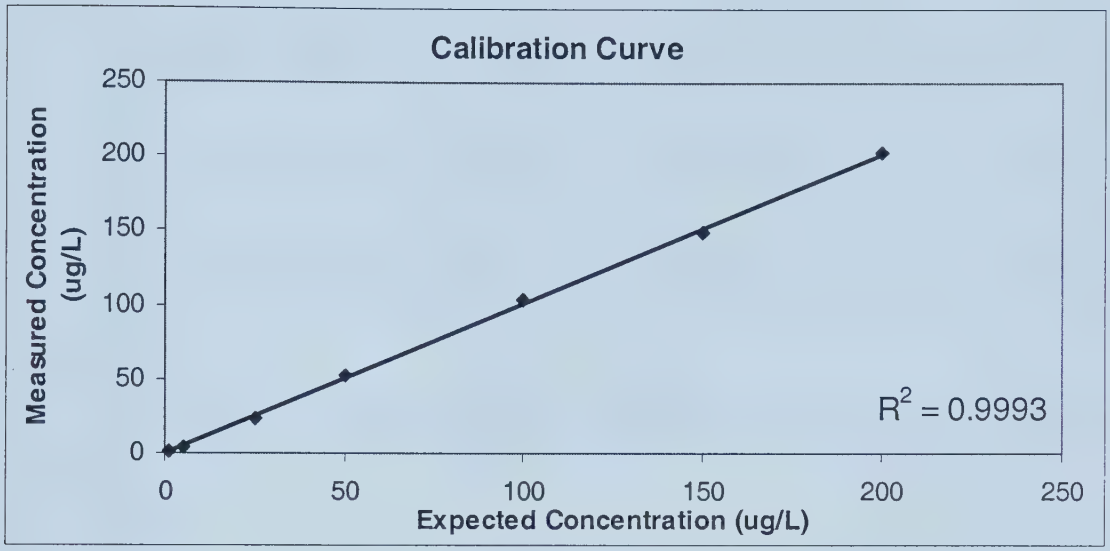
# APPENDIX 1 CALIBRATION CURVE FOR TCAA



# APPENDIX 2 CALIBRATION CURVE FOR SECONDARY ANALYSIS OF WINNIPEG WATER



# APPENDIX 3 EXCRETION DATA COLLECTED FOR EACH PARTICIPANT

WET-1

| Date | Collection Time | Elapsed Time | Sample Weight (g) | Urinary Output/Day (g) | Creatinine | | Trichloroacetic Acid (TCAA) | | TCAA Rate of Excretion | | Creatnine Adjusted TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration (mmol/L) | Absolute (g) | Concentration (ug/L) | Absolute (ug) | (ug/hr) | (ug/g/hr) | (ug/g) |
| 3-Jul | 7:30 | 8.0 | 347 | | 17.2 | 0.68 | 147.7 | 51.3 | 6.4 | 9.5 | 75.91 |
| 4-Jul | 6:00 | 7.5 | 426 | 1766 | 12.8 | 0.62 | 193.7 | 82.5 | 11.0 | 17.8 | 133.78 |
| 4-Jul | 11:00 | 5.0 | 163 | | 16.5 | 0.30 | 161.7 | 26.4 | 5.3 | 17.3 | 86.63 |
| 4-Jul | 13:30 | 2.5 | 271 | | 7.3 | 0.22 | 87.2 | 23.6 | 9.5 | 42.2 | 105.60 |
| 4-Jul | 15:30 | 2.0 | 376 | | 2.3 | 0.10 | 18.4 | 6.9 | 3.5 | 35.4 | 70.72 |
| 4-Jul | 18:00 | 2.5 | 211 | | 4.5 | 0.11 | 32.6 | 6.9 | 2.8 | 25.6 | 64.04 |
| 4-Jul | 23:00 | 5.0 | 319 | | 11.7 | 0.42 | 124.5 | 39.7 | 7.9 | 18.8 | 94.07 |
| 5-Jul | 6:00 | 7.0 | 633 | 1243 | 10.1 | 0.72 | 151.7 | 96.0 | 13.7 | 19.0 | 132.78 |
| 5-Jul | 10:00-16:00 | 10.0 | 331 | | 13.8 | 0.52 | 99.4 | 32.9 | 3.3 | 6.4 | 63.67 |
| 5-Jul | 19:45-23:00 | 7.0 | 279 | | 19.3 | 0.61 | 164.8 | 46.0 | 6.6 | 10.8 | 75.48 |
| 6-Jul | 6:00 | 7.0 | 655 | 1504 | 8.0 | 0.59 | 100.5 | 65.8 | 9.4 | 15.9 | 111.05 |
| 6-Jul | 8:45-15:00 | 9.0 | 276 | | 17.1 | 0.53 | 179.8 | 49.6 | 5.5 | 10.3 | 92.95 |
| 6-Jul | 17:30-20:30 | 5.5 | 382 | | 10.0 | 0.43 | 113.4 | 43.3 | 7.9 | 18.2 | 100.25 |
| 6-Jul | 21:30-23:45 | 3.25 | 191 | | 12.6 | 0.27 | 139.3 | 26.6 | 8.2 | 30.1 | 97.73 |
| 7-Jul | 4:30-6:00 | 6.25 | 1038 | 2138 | 3.6 | 0.42 | 74.8 | 77.6 | 12.4 | 29.4 | 183.68 |
| 7-Jul | 8:40-11:00 | 5.0 | 308 | | 9.6 | 0.33 | 127.4 | 39.2 | 7.8 | 23.5 | 117.32 |
| 7-Jul | 13:45-16:00 | 5.0 | 431 | | 7.3 | 0.36 | 85.3 | 36.8 | 7.4 | 20.7 | 103.30 |
| 7-Jul | 18:30-23:00 | 7.0 | 361 | | 14.2 | 0.58 | 125.1 | 45.2 | 6.5 | 11.1 | 77.88 |
| 8-Jul | 7:00-8:00 | 9.0 | 536 | 949 | 11.4 | 0.69 | 135.7 | 72.7 | 8.1 | 11.7 | 105.23 |
| 8-Jul | 11:00-14:00 | 6.0 | 168 | | 21.9 | 0.42 | 157.8 | 26.5 | 4.4 | 10.6 | 63.70 |
| 8-Jul | 19:30-23:45 | 9.75 | 245 | | 23.8 | 0.66 | 126.7 | 31.0 | 3.2 | 4.8 | 47.06 |
| 9-Jul | 7:00 | 7.25 | 393 | 820 | 13.0 | 0.58 | 206.2 | 81.0 | 11.2 | 19.3 | 140.22 |
| 9-Jul | 11:00-17:00 | 10.0 | 185 | | 32.2 | 0.67 | 203.5 | 37.6 | 3.8 | 5.6 | 55.87 |
| 9-Jul | 21:30-22:30 | 5.5 | 242 | | 21.2 | 0.58 | 206.8 | 50.0 | 9.1 | 15.7 | 86.23 |
| 10-Jul | 6:00 | 7.5 | 408 | 961 | 15.5 | 0.72 | 215.4 | 87.9 | 11.7 | 16.4 | 122.85 |
| 10-Jul | 9:45-12:00 | 6.0 | 156 | | 17.5 | 0.31 | 200.8 | 31.3 | 5.2 | 16.9 | 101.43 |
| 10-Jul | 15:10-16:30 | 4.5 | 99 | | 20.4 | 0.23 | 204.3 | 20.2 | 4.5 | 19.7 | 88.53 |
| 10-Jul | 18:30-22:30 | 6.0 | 298 | | 16.2 | 0.55 | 153.8 | 45.8 | 7.6 | 14.0 | 83.93 |
| 11-Jul | 6:00 | 7.5 | 945 | 1798 | 5.6 | 0.60 | 54.5 | 51.5 | 6.9 | 11.5 | 86.03 |
| 11-Jul | 9:00-12:00 | 6 | 242 | | 13.3 | 0.36 | 142.9 | 34.6 | 5.8 | 15.8 | 94.98 |

WET-1 continued

| Date | Collection Time | Elapsed Time | Sample Weight (g) | Urinary Output/Day (g) | Creatinine | | Trichloroacetic Acid (TCAA) | | TCAA Rate of Excretion | | Creatnine Adjusted TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration (mmol/L) | Absolute (g) | Concentration (ug/L) | Absolute (ug) | (ug/hr) | (ug/g/hr) | (ug/g) |
| 11-Jul | 14:40-15:40 | 3.7 | 193 | | 11.5 | 0.25 | 194.9 | 37.6 | 10.2 | 40.5 | 149.82 |
| 11-Jul | 17:45-00:00 | 8.3 | 418 | | 14.4 | 0.68 | 174.3 | 72.9 | 8.8 | 12.9 | 107.00 |
| 12-Jul | 6:00 | 6.0 | 394 | 2098 | 9.4 | 0.42 | 136.2 | 53.7 | 8.9 | 21.3 | 128.09 |
| 12-Jul | 8:00-11:30 | 5.5 | 382 | | 8.3 | 0.36 | 84.0 | 32.1 | 5.8 | 16.3 | 89.47 |
| 12-Jul | 13:30-15:30 | 4.0 | 416 | | 6.0 | 0.28 | 83.4 | 34.7 | 8.7 | 30.7 | 122.88 |
| 12-Jul | 17:00-19:30 | 4.0 | 394 | | 7.2 | 0.32 | 95.6 | 37.7 | 9.4 | 29.3 | 117.38 |
| 12-Jul | 21:30-23:00 | 3.5 | 512 | | 4.9 | 0.28 | 44.6 | 22.8 | 6.5 | 23.0 | 80.46 |
| 13-Jul | 6:00 | 7.0 | 1057 | 1783 | 5.1 | 0.61 | 40.6 | 42.9 | 6.1 | 10.1 | 70.37 |
| 13-Jul | 8:40-11:00 | 5.0 | 449 | | 5.1 | 0.26 | 73.5 | 33.0 | 6.6 | 25.5 | 127.40 |
| 13-Jul | 14:00 | 3.0 | 164 | | 10.3 | 0.19 | 126.0 | 20.7 | 6.9 | 36.0 | 108.14 |
| 13-Jul | 22:00-22:30 | 8.5 | 113 | | 42.1 | 0.54 | 151.7 | 17.1 | 2.0 | 3.7 | 31.85 |
| 14-Jul | 6:00 | 7.5 | 211 | 593 | 31.3 | 0.75 | 240.1 | 50.7 | 6.8 | 9.0 | 67.81 |
| 14-Jul | 12:15-16:30 | 10.5 | 130 | | 48.2 | 0.71 | 228.8 | 29.7 | 2.8 | 4.0 | 41.96 |
| 14-Jul | 23:20 | 6.8 | 252 | | 23.7 | 0.68 | 258.3 | 65.1 | 9.6 | 14.2 | 96.35 |
| 15-Jul | 7:00 | 7.7 | 409 | 1033 | 15.6 | 0.72 | 201.7 | 82.5 | 10.7 | 14.8 | 114.30 |
| 15-Jul | 11:30-15:00 | 8.0 | 245 | | 19.8 | 0.55 | 252.9 | 62.0 | 7.7 | 14.1 | 112.91 |
| 15-Jul | 18:45-23:15 | 8.25 | 379 | | 15.0 | 0.64 | 202.8 | 76.9 | 9.3 | 14.5 | 119.52 |
| 16-Jul | 9:30 | 10.25 | 1028 | 1787 | 6.0 | 0.70 | 104.2 | 107.1 | 10.5 | 15.0 | 153.52 |
| 16-Jul | 11:30-15:15 | 5.75 | 465 | | 11.2 | 0.59 | 118.2 | 55.0 | 9.6 | 16.2 | 93.30 |
| 16-Jul | 19:30-22:30 | 7.25 | 294 | | 14.8 | 0.49 | 195.3 | 57.4 | 7.9 | 16.1 | 116.65 |
| 17-Jul | 6:00 | 7.5 | 590 | 1902 | 8.6 | 0.57 | 104.3 | 61.5 | 8.2 | 14.3 | 107.21 |
| 17-Jul | 8:30-12:30 | 6.5 | 230 | | 14.8 | 0.39 | 141.8 | 32.6 | 5.0 | 13.0 | 84.70 |
| 17-Jul | 14:00-16:00 | 3.5 | 339 | | 6.0 | 0.23 | 61.5 | 20.8 | 6.0 | 25.9 | 90.61 |
| 17-Jul | 17:40-19:20 | 3.3 | 290 | | 7.7 | 0.25 | 52.5 | 15.2 | 4.6 | 18.3 | 60.27 |
| 17-Jul | 20:00-22:45 | 3.4 | 453 | | 6.1 | 0.31 | 18.4 | 8.3 | 2.5 | 7.8 | 26.67 |
| 18-Jul | 6:00 | 7.25 | 992 | 2082 | 4.9 | 0.55 | 33.0 | 32.7 | 4.5 | 8.2 | 59.54 |

WET-1 continued

| Date | Collection Time | Elapsed Time | Sample Weight (g) | Urinary Output/Day (g) | Creatinine | | Trichloroacetic Acid (TCAA) | | TCAA Rate of Excretion | | Creatnine Adjusted TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration (mmol/L) | Absolute (g) | Concentration (ug/L) | Absolute (ug) | (ug/hr) | (ug/g/hr) | (ug/g) |
| 18-Jul | 9:00-13:30 | 7.5 | 247 | | 16.4 | 0.46 | 93.9 | 23.2 | 3.1 | 6.7 | 50.62 |
| 18-Jul | 14:00-17:00 | 3.5 | 442 | | 5.0 | 0.25 | 37.2 | 16.4 | 4.7 | 18.8 | 65.77 |
| 18-Jul | 19:30-23:00 | 6.0 | 401 | | 11.4 | 0.52 | 78.4 | 31.4 | 5.2 | 10.1 | 60.80 |
| 19-Jul | 6:00 | 7.0 | 373 | 1598 | 12.6 | 0.53 | 77.3 | 28.8 | 4.1 | 7.7 | 54.23 |
| 19-Jul | 9:15-11:30 | 5.5 | 262 | | 8.6 | 0.25 | 51.5 | 13.5 | 2.5 | 9.6 | 52.94 |
| 19-Jul | 14:15-15:30 | 4.0 | 394 | | 8.1 | 0.36 | 50.9 | 20.1 | 5.0 | 13.9 | 55.55 |
| 19-Jul | 16:20-17:10 | 1.7 | 297 | | 2.9 | 0.10 | 4.5 | 1.3 | 0.8 | 8.1 | 13.72 |
| 19-Jul | 19:00-23:00 | 5.8 | 272 | | 13.8 | 0.42 | 69.7 | 19.0 | 3.3 | 7.7 | 44.65 |
| 20-Jul | 6:00 | 7.0 | 430 | 1205 | 11.8 | 0.57 | 59.7 | 25.7 | 3.7 | 6.4 | 44.73 |
| 20-Jul | 9:45-12:00 | 6.0 | 269 | | 11.4 | 0.35 | 66.5 | 17.9 | 3.0 | 8.6 | 51.57 |
| 20-Jul | 13:30-15:00 | 3.0 | 364 | | 5.6 | 0.23 | 39.2 | 14.3 | 4.8 | 20.6 | 61.88 |
| 20-Jul | 21:15-23:00 | 8.0 | 142 | | 32.6 | 0.52 | 66.4 | 9.4 | 1.2 | 2.3 | 18.01 |
| 21-Jul | 6:00 | 7.0 | 280 | 941 | 17.6 | 0.56 | 52.6 | 14.7 | 2.1 | 3.8 | 26.42 |
| 21-Jul | 10:45-15:00 | 9.0 | 260 | | 20.1 | 0.59 | 83.6 | 21.7 | 2.4 | 4.1 | 36.77 |
| 21-Jul | 17:00-19:30 | 4.5 | 199 | | 16.2 | 0.36 | 61.2 | 12.2 | 2.7 | 7.4 | 33.40 |
| 21-Jul | 0:15 | 4.75 | 202 | | 16.4 | 0.37 | 49.7 | 10.0 | 2.1 | 5.6 | 26.79 |
| 22-Jul | 6:00 | 5.75 | 435 | 1107 | 11.2 | 0.55 | 44.1 | 19.2 | 3.3 | 6.1 | 34.81 |
| 22-Jul | 8:45-15:30 | 9.5 | 382 | | 12.2 | 0.53 | 63.3 | 24.2 | 2.5 | 4.8 | 45.87 |
| 22-Jul | 17:30-23:00 | 7.5 | 290 | | 16.7 | 0.55 | 48.5 | 14.1 | 1.9 | 3.4 | 25.67 |
| 23-Jul | 7:40 | 8.7 | 452 | 1151 | 12.3 | 0.63 | 28.0 | 12.7 | 1.5 | 2.3 | 20.12 |
| 23-Jul | 11:00-13:30 | 5.8 | 427 | | 8.1 | 0.39 | 19.3 | 8.2 | 1.4 | 3.6 | 21.06 |
| 23-Jul | 17:15-22:30 | 9.0 | 272 | | 16.7 | 0.51 | 27.1 | 7.4 | 0.8 | 1.6 | 14.35 |
| 24-Jul | 6:00 | 7.5 | 328 | 1042 | 16.5 | 0.61 | 21.7 | 7.1 | 0.9 | 1.6 | 11.63 |
| 24-Jul | 8:00-16:00 | 10.0 | 391 | | 15.1 | 0.67 | 23.0 | 9.0 | 0.9 | 1.3 | 13.47 |

WET-1 continued

| Date | Collection Time | Elapsed Time | Sample Weight (g) | Urinary Output/Day (g) | Creatinine | | Trichloroacetic Acid (TCAA) | | TCAA Rate of Excretion | | Creatnine Adjusted TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration (mmol/L) | Absolute (g) | Concentration (ug/L) | Absolute (ug) | (ug/hr) | (ug/g/hr) | (ug/g) |
| 24-Jul | 18:30-23:00 | 7.0 | 323 | | 14.7 | 0.54 | 15.3 | 4.9 | 0.7 | 1.3 | 9.20 |
| 25-Jul | 6:00 | 7.0 | 387 | 850 | 14.3 | 0.63 | 21.2 | 8.2 | 1.2 | 1.9 | 13.11 |
| 25-Jul | 12:00-23:00 | 17.0 | 463 | | 23.8 | 1.25 | 29.4 | 13.6 | 0.8 | 0.6 | 10.92 |
| 26-Jul | 6:15 | 7.25 | 385 | | 13.5 | 0.59 | 20.0 | 7.7 | 1.1 | 1.8 | 13.10 |
| 27-Jul | No Sample | No Sample | No Sample | No Sample | No Sample | No sample | No Sample | No Sample | No Sample | No Sample | No Sample |
| 28-Jul | 6:30 | 8.5 | 317 | | 13.6 | 0.49 | 18.4 | 5.8 | 0.7 | 1.4 | 11.96 |
| 29-Jul | 7:30 | 9.0 | 306 | | 16.2 | 0.56 | 15.2 | 4.7 | 0.5 | 0.9 | 8.29 |
| 30-Jul | 8:00 | 9.5 | 477 | 878 | 12.7 | 0.69 | 20.0 | 9.5 | 1.0 | 1.5 | 13.92 |
| 30-Jul | 12:00-23:00 | 15.0 | 401 | | 23.0 | 1.04 | 18.4 | 7.4 | 0.5 | 0.5 | 7.07 |
| 31-Jul | 6:00 | 7.0 | 222 | | 20.4 | 0.51 | 34.6 | 7.7 | 1.1 | 2.1 | 14.99 |
| 1-Aug | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample |
| 2-Aug | 6:00 | 6.0 | 453 | 1423 | 8.3 | 0.43 | 5.9 | 2.7 | 0.4 | 1.0 | 6.28 |
| 2-Aug | 11:00-23:00 | 17.0 | 970 | | 9.4 | 1.03 | 10 | 9.7 | 0.6 | 0.6 | 9.40 |
| 3-Aug | 6:00 | 7.0 | 833 | 1657 | 5.5 | 0.52 | 4.9 | 4.1 | 0.6 | 1.1 | 7.88 |
| 3-Aug | 8:00-23:00 | 17.0 | 824 | | 12.7 | 1.18 | 22.3 | 18.4 | 1.1 | 0.9 | 15.52 |
| 4-Aug | 6:00 | 8.0 | 491 | | 8.4 | 0.47 | 15.7 | 7.7 | 1.0 | 2.1 | 16.52 |

WET-2

| Date | Collection Time | Time Last Urination | Elapsed Time | Sample Weight (g) | Creatinine | | Trichloroacetic Acid (TCAA) | | TCAA Rate of Excretion | | Creatnine Adjusted TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration (mmol/L) | Absolute (g) | Concentration (ug/L) | Absolute (ug) | (ug/hr) | (ug/g/hr) | (ug/g) |
| 4-Jul | 7:30 | 21:50 | 9.7 | 387 | 8.3 | 0.36 | 52.0 | 20.1 | 2.07 | 5.71 | 55.38 |
| 5-Jul | 8:05 | 23:10 | 8.75 | 270 | 10.6 | 0.32 | 61.6 | 16.6 | 1.90 | 5.87 | 51.37 |
| 6-Jul | 8:00 | 1:00 | 7.0 | 263 | 11.8 | 0.35 | 81.4 | 21.4 | 3.06 | 8.71 | 60.98 |
| 7-Jul | 7:40 | 23:30 | 8.2 | 234 | 11.4 | 0.30 | 71.0 | 16.6 | 2.03 | 6.71 | 55.06 |
| 8-Jul | 5:45 | 23:45 | 6.0 | 421 | 7.4 | 0.35 | 63.1 | 26.6 | 4.43 | 12.56 | 75.38 |
| 9-Jul | 6:45 | 23:50 | 6.9 | 162 | 3.4 | 0.06 | 29.5 | 4.8 | 0.69 | 11.12 | 76.70 |
| 10-Jul | 5:15 | 0:40 | 4.6 | 338 | 5.4 | 0.21 | 53.6 | 18.1 | 3.94 | 19.08 | 87.75 |
| 11-Jul | 5:05 | 11:20 | 5.75 | 293 | 7.0 | 0.23 | 52.5 | 15.4 | 2.68 | 11.53 | 66.30 |
| 12-Jul | 7:10 | 22:35 | 8.6 | 303 | 9.5 | 0.33 | 63.6 | 19.3 | 2.24 | 6.88 | 59.18 |
| 13-Jul | 8:05 | 23:05 | 9.0 | 197 | 2.7 | 0.06 | 36.2 | 7.1 | 0.79 | 13.17 | 118.52 |
| 14-Jul | 6:45 | 23:10 | 7.6 | 295 | 8.0 | 0.27 | 94.2 | 27.8 | 3.66 | 13.70 | 104.09 |
| 15-Jul | 8:00 | 22:40 | 9.3 | 297 | 7.3 | 0.25 | 75.1 | 22.3 | 2.40 | 9.78 | 90.94 |
| 16-Jul | 8:00 | 23:40 | 8.3 | 207 | 12.5 | 0.29 | 95.9 | 19.9 | 2.39 | 8.17 | 67.82 |
| 17-Jul | 7:05 | 23:35 | 7.5 | 270 | 7.9 | 0.24 | 54.9 | 14.8 | 1.98 | 8.19 | 61.43 |
| 18-Jul | 9:15 | 22:20 | 10.9 | 375 | 1.0 | 0.04 | 9.4 | 3.5 | 0.32 | 7.62 | 83.10 |
| 19-Jul | 5:35 | 23:05 | 6.5 | 382 | 5.6 | 0.24 | 20.8 | 7.9 | 1.22 | 5.05 | 32.83 |
| 20-Jul | 6:30 | 23:10 | 7.3 | 341 | 8.1 | 0.31 | 35.6 | 12.1 | 1.66 | 5.32 | 38.85 |
| 21-Jul | 5:45 | 23:55 | 5.8 | 333 | 6.4 | 0.24 | 18.5 | 6.2 | 1.06 | 4.41 | 25.55 |
| 22-Jul | 7:30 | 0:15 | 7.25 | 171 | 11.0 | 0.21 | 18.3 | 3.1 | 0.43 | 2.03 | 14.71 |
| 23-Jul | 9:10 | 0:15 | 8.9 | 207 | 5.6 | 0.13 | 8.4 | 1.7 | 0.20 | 1.49 | 13.26 |
| 24-Jul | 7:05 | 23:05 | 8.0 | 223 | 10.2 | 0.26 | 8.2 | 1.8 | 0.23 | 0.89 | 7.11 |
| 25-Jul | 8:15 | 0:00 | 8.25 | 207 | 12.7 | 0.30 | 15.6 | 3.2 | 0.39 | 1.32 | 10.86 |
| 26-Jul | 6:55 | 23:30 | 7.4 | 350 | 6.3 | 0.25 | 2.8 | 1.0 | 0.13 | 0.53 | 3.93 |
| 27-Jul | 8:10 | 23:40 | 8.5 | 281 | 10.1 | 0.32 | 5.0 | 1.4 | 0.17 | 0.51 | 4.38 |
| 28-Jul | 9:30 | 23:55 | 9.0 | 151 | 13.4 | 0.23 | 0.50 | 0.1 | 0.01 | 0.04 | 0.33 |

WET-3

| Date | Collection Time | Time of Last Urination | Elapsed Time (hr) | Sample Weight (g) | Creatinine Concentration (mmol/L) | Creatinine Absolute (g) | Trichloroacetic Acid (TCAA) Concentration (ug/L) | TCAA Absolute (ug) | TCAA Rate of Excretion (ug/hr) | TCAA Rate of Excretion (ug/g/hr) | Creatinine Adjusted TCAA (ug/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Jul | 7:00 | 23:30 | 7.5 | 329 | 18.4 | 0.68 | 48.3 | 15.9 | 2.1 | 3.1 | 23.2 |
| 5-Jul | 6:00 | 23:30 | 6.5 | 710 | 6.5 | 0.52 | 52.4 | 37.2 | 5.7 | 11.0 | 71.3 |
| 6-Jul | 6:00 | 23:00 | 7.0 | 675 | 9.5 | 0.73 | 57.7 | 38.9 | 5.6 | 7.7 | 53.7 |
| 7-Jul | 6:00 | 23:30 | 6.5 | 506 | 8.2 | 0.47 | 68.8 | 34.8 | 5.4 | 11.4 | 74.2 |
| 8-Jul | 6:00 | 23:30 | 6.5 | 531 | 11.7 | 0.70 | 85.9 | 45.6 | 7.0 | 10.0 | 64.9 |
| 9-Jul | 6:00 | 22:30 | 7.5 | 792 | 5.2 | 0.47 | 71.9 | 56.9 | 7.6 | 16.3 | 122.2 |
| 10-Jul | 6:00 | 0:00 | 6.0 | 579 | 9.8 | 0.64 | 65.9 | 38.2 | 6.4 | 9.9 | 59.4 |
| 11-Jul | 6:00 | 0:30 | 5.5 | 344 | 20.6 | 0.80 | 117.0 | 40.2 | 7.3 | 9.1 | 50.2 |
| 12-Jul | 6:00 | 23:30 | 6.5 | 412 | 13.8 | 0.64 | 137.7 | 56.7 | 8.7 | 13.6 | 88.2 |
| 13-Jul | 4:30 | 23:45 | 4.75 | 347 | 9.7 | 0.38 | 90.4 | 31.4 | 6.6 | 17.3 | 82.4 |
| 14-Jul | 6:00 | 23:45 | 6.25 | 380 | 22.2 | 0.95 | 196.3 | 74.6 | 11.9 | 12.5 | 78.2 |
| 15-Jul | 5:30 | 0:00 | 5.5 | 579 | 7.9 | 0.52 | 61.1 | 35.4 | 6.4 | 12.4 | 68.4 |
| 16-Jul | 5:30 | 23:45 | 5.75 | 560 | 8.0 | 0.51 | 70.3 | 39.4 | 6.8 | 13.5 | 77.7 |
| 17-Jul | 6:05 | 23:00 | 6.9 | 685 | 10.9 | 0.84 | 110.4 | 75.6 | 11.0 | 13.0 | 89.5 |
| 18-Jul | 6:00 | 0:15 | 5.75 | 550 | 8.1 | 0.50 | 39.9 | 21.9 | 3.8 | 7.6 | 43.5 |
| 19-Jul | 6:00 | 23:45 | 6.25 | 398 | 15.2 | 0.68 | 75.5 | 30.0 | 4.8 | 7.0 | 43.9 |
| 20-Jul | 6:30 | 23:00 | 7.5 | 448 | 13.9 | 0.70 | 58.1 | 26.0 | 3.5 | 4.9 | 37.0 |
| 21-Jul | 6:00 | 23:30 | 6.5 | 617 | 8.3 | 0.58 | 42.2 | 26.0 | 4.0 | 6.9 | 44.9 |
| 22-Jul | 5:30 | 23:45 | 5.75 | 348 | 21.5 | 0.85 | 67.2 | 23.4 | 4.1 | 4.8 | 27.6 |
| 23-Jul | 5:30 | 0:00 | 5.5 | 606 | 13.3 | 0.91 | 86.1 | 52.2 | 9.5 | 10.4 | 57.2 |
| 24-Jul | 8:15 | 23:30 | 8.75 | 563 | 13.4 | 0.85 | 44.1 | 24.8 | 2.8 | 3.3 | 29.1 |
| 25-Jul | 7:15 | 23:30 | 7.75 | 536 | 12.1 | 0.73 | 51.1 | 27.4 | 3.5 | 4.8 | 37.3 |
| 26-Jul | 7:15 | 23:30 | 7.75 | 498 | 9.5 | 0.54 | 23.2 | 11.6 | 1.5 | 2.8 | 21.6 |
| 27-Jul | 5:45 | 23:45 | 6.0 | 244 | 16.9 | 0.47 | 39.0 | 9.5 | 1.6 | 3.4 | 20.4 |
| 28-Jul | 6:00 | 23:00 | 7.0 | 239 | 18.3 | 0.49 | 28.3 | 6.8 | 1.0 | 2.0 | 13.7 |

WET-4

| Date | Collection Time | Time Last Urination | Elapsed Time | Sample Weight | Creatinine | | Trichloroacetic Acid (TCAA) | | TCAA Rate of Excretion | | Creatinine Adjusted TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration (mmol/L) | Absolute (g) | Concentration (ug/L) | Absolute (ug) | (ug/hr) | (ug/g/hr) | (ug/g) |
| 4-Jul | 7:15 | 23:15 | 8.0 | 363 | 13.9 | 0.57 | 254.5 | 92.4 | 11.55 | 20.23 | 161.86 |
| 5-Jul | 5:50 | 23:35 | 6.25 | 432 | 9.3 | 0.45 | 114.4 | 49.4 | 7.91 | 17.40 | 108.74 |
| 6-Jul | 6:55 | 0:20 | 6.6 | 579 | 7.1 | 0.47 | 61.8 | 35.8 | 5.42 | 11.66 | 76.95 |
| 7-Jul | 7:05 | 0:20 | 6.75 | 504 | 8.4 | 0.48 | 187.0 | 94.2 | 13.96 | 29.16 | 196.80 |
| 8-Jul | 7:50 | 0:55 | 6.9 | 478 | 9.0 | 0.49 | 107.5 | 51.4 | 7.45 | 15.30 | 105.59 |
| 9-Jul | 7:15 | 0:05 | 7.2 | 494 | 9.7 | 0.54 | 205.1 | 101.3 | 14.07 | 25.96 | 186.92 |
| 10-Jul | 7:05 | 23:05 | 8.0 | 555 | 9.0 | 0.57 | 107.9 | 59.9 | 7.49 | 13.25 | 105.98 |
| 11-Jul | 7:15 | 0:40 | 6.6 | 389 | 10.3 | 0.45 | 160.6 | 62.5 | 9.47 | 20.88 | 137.84 |
| 12-Jul | 4:25 | 23:10 | 5.25 | 430 | 7.4 | 0.36 | 172.0 | 74.0 | 14.09 | 39.14 | 205.47 |
| 13-Jul | 5:25 | 0:35 | 4.8 | 493 | 6.2 | 0.35 | 109.0 | 53.7 | 11.20 | 32.38 | 155.42 |
| 14-Jul | 7:15 | 1:25 | 5.8 | 196 | 19.3 | 0.43 | 274.6 | 53.8 | 9.28 | 21.69 | 125.78 |
| 15-Jul | 8:50 | 0:10 | 8.7 | 329 | 18.0 | 0.67 | 284.2 | 93.5 | 10.75 | 16.04 | 139.58 |
| 16-Jul | 5:35 | 0:05 | 5.5 | 437 | 8.1 | 0.40 | 107.7 | 47.1 | 8.56 | 21.37 | 117.54 |
| 17-Jul | 6:30 | 0:20 | 6.2 | 574 | 7.3 | 0.47 | 65.4 | 37.5 | 6.05 | 12.77 | 79.20 |
| 18-Jul | 6:15 | 22:35 | 7.7 | 615 | 8.2 | 0.57 | 58.4 | 35.9 | 4.66 | 8.18 | 62.96 |
| 19-Jul | 6:50 | 23:55 | 6.9 | 531 | 8.4 | 0.50 | 31.5 | 16.7 | 2.42 | 4.80 | 33.15 |
| 20-Jul | 7:05 | 0:25 | 6.7 | 423 | 10.0 | 0.48 | 58.5 | 24.7 | 3.69 | 7.72 | 51.71 |
| 21-Jul | 7:10 | 22:40 | 8.5 | 401 | 14.2 | 0.64 | 30.8 | 12.4 | 1.45 | 2.26 | 19.17 |
| 22-Jul | 8:55 | 0:15 | 8.7 | 324 | 15.2 | 0.56 | 33.2 | 10.8 | 1.24 | 2.22 | 19.31 |
| 23-Jul | 7:30 | 23:30 | 8.0 | 361 | 13.7 | 0.56 | 24.2 | 8.7 | 1.09 | 1.95 | 15.62 |
| 24-Jul | 5:25 | 23:30 | 5.9 | 509 | 7.7 | 0.44 | 12.3 | 6.3 | 1.06 | 2.39 | 14.12 |
| 25-Jul | 6:50 | 23:45 | 7.1 | 582 | 7.2 | 0.47 | 2.8 | 1.6 | 0.23 | 0.48 | 3.44 |
| 26-Jul | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample | No Sample |
| 27-Jul | 6:25 | 0:00 | 6.4 | 242 | 15.8 | 0.43 | 10.0 | 2.4 | 0.38 | 0.87 | 5.60 |
| 28-Jul | 6:10 | 23:45 | 6.4 | 378 | 10.8 | 0.46 | 3.3 | 1.2 | 0.19 | 0.42 | 2.70 |

WET-5

| Date | Collection Time | Time Last Urination | Elapsed Time | Sample Weight (g) | Creatinine Concentration (mmol/L) | Creatinine Absolute (g) | Trichloroacetic Acid (TCAA) Concentration (ug/L) | TCAA Absolute (ug) | TCAA Rate of Excretion (ug/hr) | TCAA Rate of Excretion (ug/g/hr) | Creatinine Adjusted TCAA (ug/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Jul | 7:20 | 2:00 | 5.3 | 194 | 14.6 | 0.32 | 226.5 | 43.9 | 8.29 | 4.88 | 137.14 |
| 5-Jul | 7:00 | 0:00 | 7.0 | 356 | 10.9 | 0.44 | 89.8 | 32.0 | 4.57 | 1.49 | 72.83 |
| 6-Jul | 7:05 | 0:00 | 7.1 | 223 | 13.1 | 0.33 | 119.8 | 26.7 | 3.76 | 1.60 | 80.84 |
| 7-Jul | 7:30 | 0:15 | 7.25 | 292 | 12.4 | 0.41 | 179.3 | 52.4 | 7.22 | 2.43 | 127.83 |
| 8-Jul | 6:30 | 0:00 | 6.5 | 225 | 11.9 | 0.30 | 107.0 | 24.1 | 3.70 | 1.88 | 79.49 |
| 9-Jul | 7:00 | 1:30 | 5.5 | 371 | 7.6 | 0.32 | 122.4 | 45.4 | 8.26 | 4.71 | 142.37 |
| 10-Jul | 7:30 | 0:15 | 7.25 | 494 | 8.3 | 0.46 | 65.5 | 32.4 | 4.46 | 1.33 | 69.76 |
| 11-Jul | 4:30 | 1:15 | 3.25 | 228 | 8.3 | 0.21 | 70.0 | 16.0 | 4.91 | 7.06 | 74.56 |
| 12-Jul | 7:30 | 0:45 | 6.75 | 515 | 7.0 | 0.41 | 109.0 | 56.1 | 8.32 | 3.02 | 137.65 |
| 13-Jul | 7:30 | 3:15 | 4.25 | 243 | 8.3 | 0.23 | 225.1 | 54.7 | 12.87 | 13.27 | 239.75 |
| 14-Jul | 7:50 | 0:15 | 7.25 | 432 | 9.5 | 0.46 | 55.4 | 23.9 | 3.30 | 0.98 | 51.55 |
| 15-Jul | 5:45 | 0:00 | 7.8 | 369 | 10.8 | 0.45 | 123.6 | 45.6 | 5.85 | 1.66 | 101.17 |
| 16-Jul | 7:30 | 0:50 | 5.9 | 172 | 4.5 | 0.09 | 26.6 | 4.6 | 0.78 | 1.50 | 52.26 |
| 17-Jul | 6:00 | 23:45 | 7.75 | 317 | 11.2 | 0.40 | 248.0 | 78.6 | 10.14 | 3.26 | 195.75 |
| 18-Jul | 7:30 | 23:05 | 6.9 | 294 | 9.5 | 0.32 | 55.4 | 16.3 | 2.36 | 1.08 | 51.55 |
| 19-Jul | 7:30 | 0:30 | 7.0 | 313 | 13.4 | 0.47 | 37.6 | 11.8 | 1.68 | 0.51 | 24.81 |
| 20-Jul | 7:30 | 23:50 | 7.7 | 339 | 9.8 | 0.38 | 40.4 | 13.7 | 1.78 | 0.61 | 36.44 |
| 21-Jul | 6:30 | 23:30 | 8 | 192 | 8.6 | 0.19 | 21.9 | 4.2 | 0.53 | 0.35 | 22.51 |
| 22-Jul | 6:00 | 0:10 | 6.3 | 496 | 14.8 | 0.83 | 28.1 | 13.9 | 2.21 | 0.42 | 16.78 |
| 23-Jul | 7:30 | 0:20 | 5.7 | 532 | 5.0 | 0.30 | 4.2 | 2.2 | 0.39 | 0.23 | 7.43 |
| 24-Jul | 7:30 | 23:50 | 7.7 | 366 | 3.6 | 0.15 | 3.5 | 1.3 | 0.17 | 0.14 | 8.59 |
| 25-Jul | 7:30 | 0:05 | 7.4 | 269 | 4.2 | 0.13 | 2.0 | 0.5 | 0.07 | 0.08 | 4.21 |
| 26-Jul | 7:30 | 2:00 | 5.5 | 164 | 5.1 | 0.09 | 4.1 | 0.7 | 0.12 | 0.23 | 7.11 |
| 27-Jul | 7:30 | 4:00 | 3.5 | 341 | 5.8 | 0.22 | 3.8 | 1.3 | 0.37 | 0.47 | 5.79 |
| 28-Jul | 7:30 | 23:45 | 7.75 | 376 | 4.9 | 0.21 | 2.1 | 0.8 | 0.10 | 0.06 | 3.79 |

# APPENDIX 4 INGESTION DATA COLLECTED FOR EACH PARTICIPANT

**WET-1: Ingestion Amounts (mL)**

| | 2-Jul | | 3-Jul | | 4-Jul | | 5-Jul | | 6-Jul | | 7-Jul | | 8-Jul | | 9-Jul | | 10-Jul | | 11-Jul | | 12-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | Day 8 | | Day 9 | | Day 10 | | Day 11 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 8:30 | 150 | 7:30 | 250 | 6:30 | 250 | 6:30 | 250 | 7:00 | 250 | 6:30 | 250 | 7:30 | 250 | 7:30 | 250 | 6:15 | 250 | 6:30 | 250 | 6:40 |
| | 400 | 12:15 | 250 | 10:15 | 500 | 13:00 | 250 | 9:00 | 250 | 12:30 | 250 | 9:00 | 250 | 10:00 | 500 | 11:00-13 | 250 | 7:00 | 500 | 9:10-11: | 250 | 7:40 |
| | 400 | 16:05 | 400 | 12:15 | 250 | 14:30 | 500 | 13:40-15 | 250 | 17:40 | 250 | 11:15 | 250 | 12:00 | 500 | 14:30-15 | 250 | 13:15 | 500 | 12:15-13 | 250 | 11:30 |
| | 400 | 19:00 | 250 | 14:30 | 250 | 18:30 | 500 | 16:30-17 | 250 | 18:30 | 250 | 15:30 | 250 | 14:30 | 500 | 16:30-18 | 250 | 14:15 | 500 | 18:00 | 250 | 14:00 |
| | 400 | 23:00 | 250 | 16:00 | 250 | 20:00 | 250 | 20:00 | 250 | 20:30 | 250 | 18:40 | 450 | 17:15-19 | 500 | 21:30 | 400 | 18:10 | | | 250 | 19:15 |
| | | | 250 | 17:00 | 500 | 21:45 | 250 | 23:00 | 250 | 22:30 | 250 | 21:00 | 250 | 19:40 | 250 | 22:30 | 250 | 19:45 | | | 250 | 21:30 |
| | | | 400 | 18:15 | | | | | 250 | 23:45 | | | 250 | 21:30 | | | 250 | 21:30 | | | 250 | 22:00 |
| | | | 400 | 21:00 | | | | | | | | | | | | | | | | | 250 | 22:15 |
| | | | 250 | 22:30 | | | | | | | | | | | | | | | | | | |
| Total | 1850 | 14.5 | 2600 | 15 | 2000 | 15.25 | 2000 | 16.5 | 1750 | 16.75 | 1500 | 14.5 | 1950 | 14 | 2500 | 15 | 1900 | 15.25 | 1750 | 11.5 | 2000 | 15.6 |
| Total After 17:00 | 800 | | 1300 | | 1000 | | 1000 | | 1000 | | 500 | | 950 | | 1250 | | 900 | | 500 | | 1000 | |
| Water Concentration (ug, | 97.1 | | 97.1 | | 97.1 | | 52.9 | | 50.4 | | 50.6 | | 47.6 | | 50.1 | | 51.1 | | 50.1 | | 182.5 | |
| Total Dose (ug) | 179.6 | | 252.5 | | 194.2 | | 105.8 | | 88.2 | | 75.9 | | 92.8 | | 125.3 | | 97.1 | | 87.7 | | 232.6 | |
| Daily Ingestion (ug/d) | 297.3 | | 403.9 | | 305.6 | | 153.9 | | 126.4 | | 125.6 | | 159.1 | | 200.4 | | 152.8 | | 183.0 | | 357.8 | |

| | 13-Jul | | 14-Jul | | 15-Jul | | 16-Jul | | 17-Jul | | 18-Jul | | 19-Jul | | 20-Jul | | 21-Jul | | 22-Jul | | 23-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 12 | | Day 13 | | Day 14 | | Day 15 | | Day 16 | | Day 17 | | Day 18 | | Day 19 | | Day 20 | | Day 21 | | Day 22 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 6:30 | 250 | 6:15 | 500 | 7:15 | 250 | 10:00 | 250 | 6:45 | 250 | 6:45 | 250 | 6:30 | 250 | 6:30 | 250 | 6:15 | 250 | 7:00 | 250 | 8:00 |
| | 250 | 8:00 | 350 | 7:10 | 250 | 12:00 | 500 | 15:30 | 250 | 10:30 | 250 | 8:40 | 250 | 8:00 | 250 | 8:00 | 500 | 10:00-12 | 250 | 8:30 | 250 | 11:00 |
| | 250 | 10:00 | 400 | 12:30 | 250 | 18:10 | 500 | 20:00 | 250 | 12:15 | 250 | 10:15 | 250 | 12:15 | 250 | 12:30 | 500 | 13:00-16 | 250 | 12:30 | 250 | 13:30 |
| | 250 | 12:00 | 400 | 13:30-14 | 250 | 19:00 | 250 | 21:00 | 250 | 18:30 | 250 | 12:30 | 250 | 15:10 | 250 | 13:30 | 500 | 21:00 | 250 | 14:00 | 250 | 18:00 |
| | 400 | 15:30-17 | 500 | 15:20-15 | 250 | 21:00 | | | 250 | 20:30 | 250 | 15:30 | 500 | 18:15 | 500 | 16:00-18 | 250 | 22:00 | 500 | 19:30 | 500 | 18:30 |
| | 500 | 17:30-18 | 500 | 16:20 | 250 | 22:00 | | | 250 | 22:00 | 500 | 18:30 | | | 500 | 18:30-19:30 | | | 250 | 23:00 | | |
| | 400 | 21:10 | 500 | 17:45-18:30 | | | | | | | 250 | 21:00 | | | 250 | 21:00 | | | | | | |
| | 250 | 22:30 | 500 | 19:00-19:30 | | | | | | | | | | | 250 | 23:00 | | | | | | |
| | | | 500 | 21:00 | | | | | | | | | | | | | | | | | | |
| Total | 2550 | 16 | 3900 | 10.1 | 1750 | | 1500 | | 1500 | | 2000 | | 1500 | | 2500 | | 2000 | | 1750 | | 1500 | |
| Total After 17:00 | 1150 | | 1500 | | 1000 | | 750 | | 750 | | 750 | | 500 | | 1500 | | 750 | | 750 | | 750 | |
| Water Concentration (ug, | 103.9 | | 106.7 | | | | | | | | | | | | | | | | | | | |
| Total Dose (ug) | 264.9 | | 256.1 | | | | | | | | | | | | | | | | | | | |
| Daily Ingestion (ug/d) | 397.4 | | 608.5 | | | | | | | | | | | | | | | | | | | |

| | 24-Jul | | 25-Jul | | 26-Jul | | 27-Jul | | 28-Jul | | 29-Jul | | 30-Jul | | 31-Jul | | 1-Aug | | 2-Aug | | 3-Aug | | 4-Aug | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 23 | | Day 24 | | Day 25 | | Day 26 | | Day 27 | | Day 28 | | Day 29 | | Day 30 | | Day 31 | | Day 32 | | Day 33 | | Day 34 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 500 | 6:30 | 250 | 6:30 | 250 | 6:30 | 250 | 9:00 | 250 | 11:00 | 500 | 8:30 | 250 | 6:00 | 250 | 6:30 | 500 | 7:00 | 250 | 7:00 | 250 | 7:00 | 250 | 7:00 |
| | 250 | 12:00 | 500 | 12:00 | 500 | 11:00-13 | 250 | 11:00 | 750 | 14:00-17 | 250 | 12:00 | 250 | 8:30 | 500 | 10:00-16 | 500 | 10:00 | 250 | 10:00 | 250 | 10:00 | | |
| | 250 | 15:00 | 250 | 16:15 | 250 | 19:15 | 500 | 15:30-17 | 750 | 18:00-19 | 250 | 14:00 | 250 | 10:00 | 500 | 19:30-21 | 500 | 12:30 | 250 | 12:30 | 250 | 12:30 | | |
| | 500 | 19:15 | 500 | 19:15 | 250 | 20:00 | 500 | 19:30-20 | 250 | 21:00 | 250 | 16:00 | 250 | 12:30 | 500 | 21:00-22 | 500 | 21:00 | 250 | 18:00 | 250 | 18:00 | | |
| | | | | | 250 | 21:30 | | | | | 250 | 18:45 | 250 | 16:10 | | | | | 250 | 22:30 | 250 | 22:30 | | |
| | | | | | | | | | | | 250 | 20:00 | 500 | 19:30 | | | | | | | | | | |
| | | | | | | | | | | | 250 | 21:00 | 250 | 22:30 | | | | | | | | | | |
| Total | 1500 | | 1500 | | 1500 | | 1500 | | 2000 | | 2000 | | 2000 | | 1750 | | 2000 | | 1250 | | 1250 | | 250 | |
| Total After 17:00 | 500 | | 500 | | 750 | | 1000 | | 1750 | | 750 | | 750 | | 1000 | | 500 | | 500 | | 500 | | | |

**WET-2: Ingestion Amounts (mL)**

| | 3-Jul | | 4-Jul | | 5-Jul | | 6-Jul | | 7-Jul | | 8-Jul | | 9-Jul | | 10-Jul | | 11-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | Day 8 | | Day 9 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 360 | 8:45 | 250 | 11:40 | 350 | 10:15 | 250 | 13:20 | 250 | 10:50 | 300 | 10:15 | 300 | 10:45 | 250 | 12:15 | 200 | 10:45 |
| | 250 | 9:40 | 250 | 16:35 | 200 | 15:05 | 250 | 17:05 | 250 | 17:10 | 200 | 17:30 | 200 | 15:45 | 200 | 19:05 | 300 | 16:45 |
| | 390 | 21:15 | 325 | 18:10 | 300 | 19:15 | 300 | 19:35 | 300 | 18:50 | 300 | 19:05 | 300 | 19:30 | 300 | 20:15 | 250 | 18:45 |
| | 350 | 0:35 | 275 | 19:00 | 300 | 19:35 | 250 | 20:05 | 200 | 20:05 | 250 | 22:40 | 300 | 21:30 | 300 | 21:30 | 300 | 19:45 |
| | | | 250 | 20:50 | 350 | 20:55 | 300 | 23:00 | 300 | 22:05 | 300 | 11:15 | 300 | 22:15 | 200 | 22:45 | 300 | 22:20 |
| | | | | | | | 150 | 23:45 | 200 | 22:35 | 150 | 0:05 | 100 | 22:40 | | | 150 | 23:40 |
| Total | 1350 | 11.8 | 1350 | 9.2 | 1500 | 10.7 | 1500 | 10.4 | 1500 | 11.75 | 1500 | 13.8 | 1500 | 11.9 | 1250 | 10.5 | 1500 | 12.9 |
| Total After 17:00 | 740 | | 850 | | 950 | | 1250 | | 1250 | | 1200 | | 1000 | | 1000 | | 1000 | |
| Water Concentration (ug | 97.1 | | 97.1 | | 52.9 | | 50.4 | | 50.6 | | 47.6 | | 50.1 | | 51.1 | | 50.1 | |
| Total Dose (ug) | 131.1 | | 131.1 | | 79.4 | | 75.6 | | 75.9 | | 71.4 | | 75.2 | | 63.9 | | 75.2 | |
| Daily ingestion (ug/d) | 266.6 | | 342.0 | | 178.0 | | 174.5 | | 155.0 | | 124.2 | | 151.6 | | 146.0 | | 139.8 | |

| | 12-Jul | | 13-Jul | | 14-Jul | | 15-Jul | | 16-Jul | | 17-Jul | | 18-Jul | | 19-Jul | | 20-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 10 | | Day 11 | | Day 12 | | Day 13 | | Day 14 | | Day 15 | | Day 16 | | Day 17 | | Day 18 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 12:40 | 250 | 8:00 | 200 | 7:55 | 250 | 8:05 | 250 | 10:30 | 250 | 10:30 | 250 | 6:45 | 250 | 12:40 | 250 | 7:45 |
| | 250 | 16:45 | 250 | 12:35 | 300 | 10:15 | 250 | 13:40 | 750 | 12:00 | 250 | 15:40 | 250 | 13:00 | 250 | 17:30 | 250 | 12:35 |
| | 300 | 17:50 | 250 | 17:50 | 300 | 19:10 | 250 | 18:40 | 250 | 17:00 | 250 | 18:35 | 200 | 17:45 | 250 | 19:00 | 300 | 17:10 |
| | 300 | 19:15 | 250 | 19:35 | 300 | 20:00 | 250 | 20:15 | 300 | 21:55 | 250 | 20:10 | 250 | 19:30 | 250 | 20:05 | 250 | 19:25 |
| | 300 | 21:35 | 250 | 22:25 | 300 | 21:40 | 250 | 21:25 | 250 | 22:35 | 300 | 20:45 | 300 | 20:45 | 300 | 21:15 | 250 | 21:30 |
| | 100 | 23:10 | 250 | 23:50 | 100 | 22:20 | 250 | 22:30 | 200 | 23:30 | 200 | 22:40 | | | | | 200 | 22:00 |
| Total | 1500 | 10.5 | 1500 | 15.8 | 1500 | 14.4 | 1500 | 5.6 | 2000 | | 1500 | | 1250 | | 1300 | | 1500 | |
| Total After 17:00 | 1000 | | 1000 | | 1000 | | 1000 | | 1000 | | 1000 | | 750 | | 1050 | | 1000 | |
| Water Concentration (ug | 182.5 | | 103.9 | | 106.7 | | 97.1 | | | | | | | | | | | |
| Total Dose (ug) | 207.6 | | 155.9 | | 160.1 | | 48.6 | | | | | | | | | | | |
| Daily ingestion (ug/d) | 474.4 | | 236.7 | | 266.8 | | 208.1 | | | | | | | | | | | |

| | 21-Jul | | 22-Jul | | 23-Jul | | 24-Jul | | 25-Jul | | 26-Jul | | 27-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 19 | | Day 20 | | Day 21 | | Day 22 | | Day 23 | | Day 24 | | Day 25 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 9:15 | 300 | 11:50 | 300 | 12:00 | 250 | 10:30 | 250 | 11:00 | 250 | 9:10 | 250 | 11:10 |
| | 300 | 12:10 | 200 | 12:35 | 200 | 15:00 | 300 | 17:10 | 250 | 17:50 | 250 | 12:50 | 250 | 13:15 |
| | 200 | 17:30 | 250 | 19:20 | 250 | 18:10 | 250 | 19:45 | 250 | 20:00 | 250 | 18:30 | 300 | 19:20 |
| | 250 | 18:40 | 250 | 21:50 | 250 | 20:15 | 300 | 21:10 | 250 | 21:45 | 250 | 20:00 | 300 | 20:50 |
| | 350 | 21:05 | 250 | 23:00 | 250 | 21:50 | 200 | 23:30 | 250 | 22:50 | 250 | 22:00 | 300 | 21:15 |
| | 200 | 21:45 | | | 250 | 23:05 | | | | | 250 | 23:20 | 100 | 23:00 |
| Total | 1550 | | 1250 | | 1500 | | 1300 | | 1250 | | 1500 | | 1500 | |
| Total After 17:00 | 1000 | | 750 | | 1000 | | 1050 | | 1000 | | 1000 | | 1000 | |

**WET-3: Ingestion Amounts (mL)**

| | 3-Jul | | 4-Jul | | 5-Jul | | 6-Jul | | 7-Jul | | 8-Jul | | 9-Jul | | 10-Jul | | 11-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | Day 8 | | Day 9 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 500 | 8:00 | 500 | 8:00 | 500 | 9:00 | 500 | 8:00 | 500 | 10:00 | 500 | 8:00 | 500 | 11:00 | 500 | 6:00 | 500 | 7:00 |
| | 500 | 11:00 | 500 | 11:00 | 500 | 12:00 | 500 | 10:00 | 500 | 12:00 | 500 | 10:00 | 500 | 12:00 | 500 | 9:00 | 500 | 10:00 |
| | 500 | 13:00 | 500 | 14:00 | 500 | 14:00 | 500 | 12:00 | 500 | 15:00 | 500 | 13:00 | 500 | 15:00 | 500 | 12:30 | 500 | 12:00 |
| | 500 | 18:00 | 500 | 18:00 | 500 | 18:00 | 500 | 14:00 | 500 | 18:30 | 500 | 17:00 | 500 | 18:30 | 500 | 15:30 | 500 | 14:30 |
| | 500 | 20:00 | 500 | 21:00 | 500 | 20:00 | 500 | 18:00 | 500 | 20:30 | 500 | 19:30 | 500 | 19:30 | 500 | 17:00 | 500 | 17:00 |
| | | | | | 500 | 22:00 | 500 | 20:30 | | | | | | | 500 | 20:00 | 500 | 19:00 |
| | | | | | | | | | | | | | | | 500 | 22:00 | 500 | 21:00 |
| Total | 2500 | 12 | 2500 | 13 | 3000 | 13 | 3000 | 12.5 | 2500 | 10.5 | 2500 | 11.5 | 2500 | 8.5 | 3500 | 16 | 3500 | 14 |
| Total After 17:00 | 1000 | | 1000 | | 1500 | | 1000 | | 1000 | | 1000 | | 1000 | | 1500 | | 1500 | |
| Water Concentration (ug | 97.1 | | 97.1 | | 52.9 | | 50.4 | | 50.6 | | 47.6 | | 50.1 | | 51.1 | | 50.1 | |
| Total Dose (ug) | 242.8 | | 242.8 | | 158.7 | | 151.2 | | 126.5 | | 119.0 | | 125.3 | | 178.9 | | 175.4 | |
| Daily Ingestion (ug/d) | 485.5 | | 448.2 | | 293.0 | | 290.3 | | 289.1 | | 248.3 | | 353.6 | | 268.3 | | 300.6 | |

| | 12-Jul | | 13-Jul | | 14-Jul | | 15-Jul | | 16-Jul | | 17-Jul | | 18-Jul | | 19-Jul | | 20-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 10 | | Day 11 | | Day 12 | | Day 13 | | Day 14 | | Day 15 | | Day 16 | | Day 17 | | Day 18 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 500 | 8:00 | 500 | 7:30 | 500 | 8:00 | 500 | 9:00 | 500 | 9:00 | 500 | 8:30 | 500 | 8:00 | 500 | 8:00 | 500 | 8:00 |
| | 500 | 11:30 | 500 | 9:30 | 500 | 10:30 | 500 | 11:00 | 500 | 11:30 | 500 | 11:00 | 500 | 10:30 | 500 | 10:30 | 500 | 10:45 |
| | 500 | 12:30 | 500 | 12:00 | 500 | 12:00 | 500 | 12:00 | 500 | 12:30 | 500 | 12:45 | 500 | 12:15 | 500 | 12:00 | 500 | 12:00 |
| | 500 | 14:30 | 500 | 15:00 | 500 | 14:30 | 500 | 15:00 | 500 | 15:30 | 500 | 16:00 | 500 | 14:30 | 500 | 15:30 | 500 | 13:30 |
| | 500 | 17:00 | 500 | 17:30 | 500 | 17:00 | 500 | 17:30 | 500 | 17:00 | 500 | 17:30 | 500 | 17:30 | 500 | 17:30 | 500 | 18:00 |
| | 500 | 18:30 | 500 | 19:30 | 500 | 20:00 | 500 | 20:00 | 500 | 19:00 | 500 | 19:00 | 500 | 19:00 | 500 | 20:00 | 500 | 20:30 |
| | 500 | 20:00 | 500 | 21:30 | 500 | 22:00 | 500 | 22:00 | 500 | 21:30 | 500 | 21:30 | 500 | 21:00 | 500 | 22:30 | 500 | 21:30 |
| | | | | | | | 500 | 23:30 | | | | | 500 | 23:00 | | | 500 | 23:30 |
| Total | 3500 | 12 | 3500 | 14 | 3500 | 14 | 4000 | 6 | 3500 | | 3500 | | 4000 | | 3500 | | 4000 | |
| Total After 17:00 | 1500 | | 1500 | | 1500 | | 2000 | | 1500 | | 1500 | | 2000 | | 1500 | | 2000 | |
| Water Concentration (ug | 182.5 | | 103.9 | | 106.7 | | 97.1 | | | | | | | | | | | |
| Total Dose (ug) | 374.0 | | 363.7 | | 373.5 | | 194.2 | | | | | | | | | | | |
| Daily Ingestion (ug/d) | 747.9 | | 623.4 | | 640.2 | | 776.8 | | | | | | | | | | | |

| | 21-Jul | | 22-Jul | | 23-Jul | | 24-Jul | | 25-Jul | | 26-Jul | | 27-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 19 | | Day 20 | | Day 21 | | Day 22 | | Day 23 | | Day 24 | | Day 25 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 500 | 8:00 | 500 | 9:00 | 500 | 9:00 | 500 | 8:00 | 500 | 8:00 | 500 | 8:00 | 500 | 8:00 |
| | 500 | 10:30 | 500 | 11:30 | 500 | 11:30 | 500 | 10:00 | 500 | 10:30 | 500 | 10:30 | 500 | 10:30 |
| | 500 | 12:00 | 500 | 12:30 | 500 | 13:00 | 500 | 12:30 | 500 | 13:00 | 500 | 12:00 | 500 | 12:45 |
| | 500 | 14:30 | 500 | 16:00 | 500 | 15:00 | 500 | 15:00 | 500 | 16:30 | 500 | 14:00 | 500 | 14:30 |
| | 500 | 17:00 | 500 | 18:30 | 500 | 17:30 | 500 | 18:30 | 500 | 20:00 | 500 | 17:30 | 500 | 17:45 |
| | 500 | 19:30 | 500 | 19:30 | 500 | 21:00 | 500 | 20:00 | 500 | 22:00 | 500 | 21:30 | 500 | 19:30 |
| | 500 | 21:00 | 500 | 23:00 | 500 | 22:30 | 500 | 21:00 | | | | | 500 | 21:30 |
| | | | | | | | | | | | | | 500 | 23:00 |
| Total | 3500 | | 3500 | | 3500 | | 3500 | | 3000 | | 3000 | | 4000 | |
| Total After 17:00 | 1500 | | 1500 | | 1500 | | 1500 | | 1000 | | 1000 | | 2000 | |

**WET-4: Ingestion Amounts (mL)**

| | 3-Jul | | 4-Jul | | 5-Jul | | 6-Jul | | 7-Jul | | 8-Jul | | 9-Jul | | 10-Jul | | 11-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | Day 8 | | Day 9 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 400 | 12:00 | 250 | 8:30 | 250 | 8:20 | 250 | 7:50 | 250 | 7:40 | 250 | 8:20 | 250 | 9:45 | 200 | 7:30 | 250 | 7:50 |
| | 400 | 13:40 | 500 | 10:00 | 500 | 9:50 | 500 | 30-11:30 | 500 | 10-10:30 | 400 | 9:30 | 400 | 13:15 | 200 | 8:00 | 200 | 10:00 |
| | 500 | 15:00 | 500 | 12:45 | 500 | 15:00 | 500 | 14:00 | 500 | 00-14:00 | 400 | 12:00 | 400 | 17:35 | 500 | 11:20 | 300 | 11:40 |
| | 400 | 18:00 | 250 | 15:30 | 250 | 16:15 | 400 | 16:40 | 400 | 18:30 | 400 | 16:00 | 400 | 21:15 | 500 | 14:10 | 400 | 15:20 |
| | 400 | 19:30 | 400 | 18:20 | 400 | 18:15 | 400 | 21:00 | 250 | 20:20 | 400 | 17:50 | 200 | 23:00 | 200 | 16:40 | 400 | 20:30 |
| | 250 | 23:00 | 400 | 22:00 | 400 | 22:00 | 250 | 23:20 | 250 | 21:50 | 400 | 20:15 | | | 400 | 18:00 | 400 | 22:40 |
| | | | 200 | 23:30 | 200 | 23:30 | | | 400 | 23:30 | 150 | 0:00 | | | 400 | 21:30 | 100 | 23:10 |
| | | | | | | | | | | | | | | | 250 | 22:55 | | |
| Total | 2350 | 11 | 2500 | 15 | 2500 | 15.2 | 2300 | 15.5 | 2550 | 15.8 | 2400 | 15.7 | 1650 | 13.25 | 2650 | 15.4 | 2050 | 15.3 |
| Total After 17:00 | 1050 | | 1000 | | 1000 | | 650 | | 1300 | | 950 | | 1000 | | 1050 | | 900 | |
| Water Concentration (ug | 97.1 | | 97.1 | | 52.9 | | 50.4 | | 50.6 | | 47.6 | | 50.1 | | 51.1 | | 50.1 | |
| Total Dose (ug) | 228.2 | | 242.8 | | 132.3 | | 115.9 | | 129.0 | | 114.2 | | 82.7 | | 135.4 | | 102.7 | |
| Daily ingestion (ug/d) | 497.9 | | 388.4 | | 208.8 | | 179.5 | | 196.0 | | 174.6 | | 149.7 | | 211.0 | | 161.1 | |

| | 12-Jul | | 13-Jul | | 14-Jul | | 15-Jul | | 16-Jul | | 17-Jul | | 18-Jul | | 19-Jul | | 20-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 10 | | Day 11 | | Day 12 | | Day 13 | | Day 14 | | Day 15 | | Day 16 | | Day 17 | | Day 18 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 8:20 | 400 | 7:30 | 400 | 7:45 | 250 | 12:10 | 250 | 7:55 | 250 | 8:05 | 250 | 7:40 | 250 | 7:35 | 325 | 7:35 |
| | 500 | 13:10 | 250 | 9:45 | 250 | 12:00 | 400 | 13:15 | 200 | 8:30 | 500 | 12:00 | 500 | 9:30 | 250 | 8:50 | 250 | 11:10 |
| | 500 | 15:50-16 | 500 | 13:30 | 250 | 13:15 | 200 | 16:45 | 300 | 10:30 | 400 | 13:50 | 500 | 12:30 | 500 | 12:05 | 500 | 16:35 |
| | 50 | 17:00 | 500 | 15:15-16 | 250 | 15:35 | 400 | 18:45 | 400 | 13:15 | 400 | 20:10 | 250 | 14:45 | 250 | 16:25 | 400 | 18:20 |
| | 400 | 18:30-19 | 500 | 17:00-18 | 400 | 18:00 | 100 | 19:00 | 300 | 15:00 | 200 | 22:30 | 400 | 19:00-20 | 400 | 19:00 | 400 | 22:25 |
| | 400 | 21:20 | 400 | 19:30 | 200 | 20:20 | 400 | 21:45 | 500 | 18:20 | | | 400 | 22:00 | 500 | 21:30 | | |
| | 250 | 0:15 | 400 | 21:40 | 400 | 23:10 | 200 | 23:20 | 500 | 23:55 | | | 200 | 23:00 | 200 | 23:50 | | |
| | | | 250 | 22:25 | | | | | | | | | | | | | | |
| Total | 2350 | 15.9 | 3200 | 14.9 | 2150 | 15.4 | 1950 | 4.6 | 2450 | | 1750 | | 2300 | | 2350 | | 1875 | |
| Total After 17:00 | 1100 | | 1550 | | 1000 | | 1100 | | 1000 | | 600 | | 1000 | | 1100 | | 800 | |
| Water Concentration (ug | 182.5 | | 103.9 | | 106.7 | | 97.1 | | | | | | | | | | | |
| Total Dose (ug) | 263.4 | | 332.5 | | 229.4 | | 82.5 | | | | | | | | | | | |
| Daily ingestion (ug/d) | 397.5 | | 535.5 | | 357.5 | | 430.6 | | | | | | | | | | | |

| | 21-Jul | | 22-Jul | | 23-Jul | | 24-Jul | | 25-Jul | | 26-Jul | | 27-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 19 | | Day 20 | | Day 21 | | Day 22 | | Day 23 | | Day 24 | | Day 25 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 8:00 | 250 | 10:45 | 250 | 9:40 | 300 | 7:35 | 250 | 7:30 | 300 | 7:35 | 400 | 7:15 |
| | 500 | 9:45 | 500 | 11:30 | 200 | 11:00 | 250 | 8:45 | 250 | 10:00 | 300 | 8:55 | 250 | 00-10:00 |
| | 250 | 12:20 | 500 | 14:00 | 400 | 13:00 | 500 | 10:45-11 | 250 | 11:00 | 300 | 12:40 | 500 | 15:30 |
| | 275 | 16:15 | 250 | 17:15 | 400 | 15:00 | 500 | 15:40 | 500 | 13:50 | 400 | 18:00 | 400 | 17:45 |
| | 400 | 17:55 | 400 | 18:30 | 400 | 19:00 | 400 | 18:00 | 400 | 18:00 | 400 | 19:00 | 200 | 18:30 |
| | 400 | 20:20 | 400 | 22:00 | 200 | 23:30 | 400 | 20:30 | 500 | 18:45 | 325 | 23:35 | 200 | 20:30 |
| | 275 | 22:50 | | | | | 200 | 21:00 | 200 | 23:20 | | | 250 | 23:25 |
| | | | | | | | 200 | 23:35 | | | | | | |
| Total | 2350 | | 2300 | | 1850 | | 2750 | | 2350 | | 2025 | | 2200 | |
| Total After 17:00 | 1075 | | 1050 | | 600 | | 1200 | | 1100 | | 1125 | | 1050 | |

**WET-5: Ingestion Amounts (mL)**

| | 3-Jul | | 4-Jul | | 5-Jul | | 6-Jul | | 7-Jul | | 8-Jul | | 9-Jul | | 10-Jul | | 11-Jul | | 12-Jul | | 13-Jul | | 14-Jul | | 15-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | Day 8 | | Day 9 | | Day 10 | | Day 11 | | Day 12 | | Day 13 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 250 | 10:30 | 250 | 7:25 | 400 | 7:05 | 400 | 7:10 | 200 | 8:40 | 400 | 10:15 | 400 | 7:10 | 300 | 7:30 | 300 | 7:30 | 200 | 7:30 | 200 | 7:30 | 200 | 7:30 | 250 | 7:50 |
| | 275 | 11:45 | 250 | 10:25 | 250 | 9:25 | 350 | 8:30 | 200 | 10:00 | 500 | 14:00 | 250 | 9:45 | 400 | 10:00 | 250 | 9:45 | 300 | 10:00 | 250 | 8:45 | 200 | 9:00 | 250 | 9:30 |
| | 350 | 12:30 | 200 | 13:00 | 250 | 10:40 | 350 | 10:30 | 300 | 11:30 | 250 | 15:00 | 250 | 11:00 | 200 | 11:30 | 250 | 11:30 | 250 | 12:00 | 100 | 9:10 | 200 | 10:00 | 200 | 13:30 |
| | 250 | 13:50 | 500 | 16:00 | 250 | 12:00 | 400 | 12:30 | 250 | 12:15 | 100 | 15:10 | 350 | 11:40 | 300 | 12:30 | 500 | 13:15 | 250 | 14:00 | 300 | 10:30 | 200 | 11:30 | 250 | 18:20 |
| | 250 | 15:20 | 450 | 19:30 | 450 | 13:00 | 150 | 14:15 | 400 | 14:00 | 250 | 18:25 | 400 | 13:30 | 300 | 13:00 | 250 | 15:05 | 250 | 14:25 | 400 | 14:15 | 400 | 12:30 | 250 | 20:00 |
| | 250 | 16:45 | 450 | 21:30 | 300 | 14:30 | 250 | 15:00 | 400 | 16:25 | 400 | 19:20 | 500 | 15:30 | 300 | 14:00 | 300 | 16:00 | 250 | 15:20 | 400 | 16:00 | 250 | 13:30 | 450 | 20:30 |
| | 250 | 17:50 | | | 200 | 16:30 | 150 | 16:10 | 250 | 19:15 | 250 | 20:30 | 450 | 18:30 | 300 | 15:15 | 450 | 19:15 | 250 | 15:55 | 300 | 17:45 | 250 | 14:45 | 250 | 22:30 |
| | 250 | 18:45 | | | 100 | 16:50 | 350 | 17:30 | 250 | 22:30 | 250 | 21:15 | 250 | 21:40 | 300 | 16:40 | 100 | 19:40 | 300 | 16:45 | 250 | 18:00 | 250 | 16:00 | 250 | 23:10 |
| | 250 | 21:30 | | | 400 | 18:00 | 250 | 21:00 | 250 | 23:20 | 250 | 22:00 | 350 | 23:00 | 550 | 18:00 | 250 | 21:30 | 600 | 18:00 | 400 | 19:00 | 400 | 18:00 | 150 | 0:05 |
| | 250 | 22:00 | | | 250 | 20:30 | 250 | 22:00 | 250 | 0:15 | 250 | 23:30 | | | 500 | 20:05 | 250 | 23:00 | 350 | 18:55 | 250 | 20:30 | 250 | 21:00 | | |
| | | | | | 500 | 22:00 | | | | | | | | | 500 | 23:40 | 250 | 0:00 | 400 | 21:30 | 250 | 21:30 | 250 | 23:00 | | |
| | | | | | | | | | | | | | | | | | | | 400 | 22:30 | 250 | 23:10 | 100 | 0:20 | | |
| | | | | | | | | | | | | | | | | | | | 200 | 23:20 | | | | | | |
| Total | 2625 | 11.5 | 2100 | 15.1 | 3350 | 14.9 | 2900 | 14.8 | 2750 | 15.6 | 2900 | 13.25 | 3200 | 15.8 | 3950 | 16.2 | 3150 | 16.5 | 4000 | 15.8 | 3350 | 15.7 | 2950 | 16.8 | 2300 | 5.7 |
| Total After 17:00 | 1000 | | 900 | | 1150 | | 850 | | 1000 | | 1650 | | 1050 | | 1550 | | 1300 | | 1950 | | 1700 | | 1000 | | 1600 | |
| Water Concentration (ug | 97.1 | | 97.1 | | 52.9 | | 50.4 | | 50.6 | | 47.6 | | 50.1 | | 51.1 | | 50.1 | | 182.5 | | 103.9 | | 106.7 | | 97.1 | |
| Total Dose (ug) | 254.9 | | 203.9 | | 177.2 | | 146.2 | | 139.2 | | 138.0 | | 160.3 | | 201.8 | | 157.8 | | 458.6 | | 348.1 | | 314.8 | | 68.0 | |
| Daily Ingestion (ug/d) | 531.9 | | 324.1 | | 285.4 | | 237.0 | | 214.1 | | 250.0 | | 243.5 | | 299.0 | | 229.5 | | 696.6 | | 532.1 | | 449.7 | | 286.2 | |

| | 16-Jul | | 17-Jul | | 18-Jul | | 19-Jul | | 20-Jul | | 21-Jul | | 22-Jul | | 23-Jul | | 24-Jul | | 25-Jul | | 26-Jul | | 27-Jul | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 14 | | Day 15 | | Day 16 | | Day 17 | | Day 18 | | Day 19 | | Day 20 | | Day 21 | | Day 22 | | Day 23 | | Day 24 | | Day 25 | |
| | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time | Amount | Time |
| | 300 | 5:45 | 200 | 7:30 | 450 | 6:30 | 300 | 7:30 | 250 | 7:30 | 200 | 7:30 | 200 | 6:30 | 250 | 6:00 | 250 | 7:30 | 250 | 7:30 | 200 | 7:30 | 200 | 7:30 |
| | 200 | 9:00 | 200 | 8:30 | 500 | 9:00 | 250 | 9:00 | 250 | 8:00 | 250 | 8:30 | 250 | 8:45 | 250 | 8:15 | 400 | 11:15 | 250 | 9:00 | 200 | 8:40 | 350 | 8:45 |
| | 200 | 11:50 | 400 | 10:30 | 500 | 11:30 | 250 | 10:30 | 400 | 10:30 | 400 | 12:05 | 250 | 10:15 | 150 | 9:30 | 400 | 14:00 | 400 | 10:10 | 350 | 9:45 | 400 | 12:20 |
| | 250 | 14:20 | 400 | 12:15 | 500 | 13:00 | 450 | 12:15 | 250 | 12:30 | 400 | 13:50 | 250 | 10:45 | 150 | 9:45 | 400 | 15:00 | 400 | 12:50 | 200 | 10:30 | 400 | 14:30 |
| | 200 | 16:00 | 400 | 16:45 | 500 | 15:30 | 400 | 14:40 | 450 | 13:45 | 400 | 15:45 | 500 | 13:30 | 300 | 10:30 | 400 | 16:45 | 400 | 15:30 | 500 | 11:30 | 400 | 15:40 |
| | 200 | 18:15 | 200 | 17:45 | 250 | 18:00 | 450 | 15:30 | 200 | 14:40 | 250 | 16:30 | 450 | 15:45 | 150 | 12:30 | 500 | 18:00 | 200 | 16:00 | 300 | 13:15 | 300 | 17:00 |
| | 250 | 23:00 | 400 | 18:05 | 150 | 19:00 | 400 | 17:45 | 400 | 17:30 | 500 | 17:45 | 250 | 17:30 | 250 | 13:20 | 500 | 19:45 | 400 | 17:15 | 450 | 13:30 | 250 | 18:30 |
| | | | 200 | 20:30 | 250 | 20:20 | 450 | 22:10 | 400 | 19:30 | 500 | 22:30 | 500 | 20:30 | 250 | 14:10 | 200 | 23:30 | 450 | 18:30 | 350 | 14:30 | 300 | 20:00 |
| | | | 250 | 21:20 | | | 400 | 23:45 | 500 | 20:45 | 200 | 0:10 | 500 | 23:30 | 250 | 17:00 | | | 150 | 19:05 | 250 | 15:50 | 350 | 22:15 |
| | | | 450 | 22:00 | | | | | 500 | 22:30 | | | | | 250 | 18:30 | | | 500 | 21:30 | 250 | 16:45 | 200 | 23:45 |
| | | | 250 | 23:00 | | | | | 200 | 23:30 | | | | | 500 | 19:30 | | | | | 250 | 17:30 | | |
| | | | | | | | | | | | | | | | 150 | 20:30 | | | | | 350 | 19:00 | | |
| | | | | | | | | | | | | | | | 500 | 21:45 | | | | | 350 | 20:45 | | |
| | | | | | | | | | | | | | | | 150 | 22:30 | | | | | 350 | 22:30 | | |
| | | | | | | | | | | | | | | | 500 | 23:45 | | | | | | | | |
| Total | 1600 | | 3350 | | 3100 | | 3350 | | 3800 | | 3100 | | 3150 | | 4050 | | 3050 | | 3400 | | 4350 | | 3150 | |
| Total After 17:00 | 450 | | 1750 | | 1250 | | 1250 | | 2000 | | 1200 | | 1250 | | 2300 | | 1200 | | 1500 | | 1300 | | 1400 | |

# APPENDIX 5 DATA USED FOR CORELATIONAL ANALYSIS

| | WET-1 | | | WET-2 | | | WET-3 | | | WET-4 | | | WET-5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Day | TCAA Dose (µg/d) | FMU TCAA Excretion (µg/d) | Expected TCAA Excretion (µg/d) | TCAA Dose (µg/d) | FMU TCAA Excretion (µg/d) | Expected TCAA Excretion (µg/d) | TCAA Dose (µg/d) | FMU TCAA Excretion (µg/d) | Expected TCAA Excretion (µg/d) | TCAA Dose (µg/d) | FMU TCAA Excretion (µg/d) | Expected TCAA Excretion (µg/d) | TCAA Dose (µg/d) | FMU TCAA Excretion (µg/d) | Expected TCAA Excretion (µg/d) |
| 0 | 297.3 | | | 266.6 | | | 485.5 | | | 497.9 | | | 531.9 | | |
| 1 | 403.9 | 153.8 | | 342.0 | 49.8 | | 448.2 | 50.9 | | 388.4 | 277.2 | | 324.1 | 199.0 | |
| 2 | 305.6 | 264.1 | | 178.0 | 45.6 | | 293.0 | 137.4 | | 208.8 | 189.8 | | 285.4 | 109.6 | |
| 3 | 153.9 | 329.2 | | 174.5 | 73.4 | 146.4 | 290.3 | 133.5 | | 179.5 | 130.1 | | 237.0 | 90.3 | |
| 4 | 126.4 | 225.7 | | 155.0 | 48.6 | 126.8 | 289.1 | 128.5 | | 196.0 | 335.1 | 137.0 | 214.1 | 173.3 | 212.3 |
| 5 | 125.6 | 298.1 | | 124.2 | 106.3 | 99.7 | 248.3 | 168.4 | | 174.6 | 178.7 | 113.1 | 250.0 | 88.9 | 189.3 |
| 6 | 159.1 | 194.0 | | 151.6 | 16.6 | 100.8 | 353.6 | 182.2 | | 149.7 | 337.7 | 95.3 | 243.5 | 198.2 | 182.9 |
| 7 | 200.4 | 268.3 | | 146.0 | 94.5 | 98.2 | 268.3 | 152.6 | | 211.0 | 179.7 | 100.6 | 299.0 | 107.1 | 193.9 |
| 8 | 152.8 | 281.2 | | 139.8 | 64.2 | 96.0 | 300.6 | 175.6 | | 161.1 | 227.2 | 96.6 | 229.5 | 117.9 | 188.3 |
| 9 | 183.0 | 164.8 | 106.5 | 474.4 | 53.8 | 202.0 | 747.9 | 209.5 | | 397.5 | 338.1 | 136.2 | 696.6 | 199.6 | 298.9 |
| 10 | 357.8 | 214.7 | 119.9 | 236.7 | 19.0 | 180.4 | 623.4 | 158.5 | | 535.5 | 268.7 | 191.5 | 532.1 | 308.9 | 340.5 |
| 11 | 397.4 | 147.1 | 132.8 | 266.8 | 87.8 | 189.8 | 640.2 | 286.4 | 257.2 | 357.5 | 222.7 | 267.6 | 449.7 | 79.2 | 352.5 |
| 12 | 608.5 | 162.1 | 166.6 | 208.1 | 57.6 | 176.6 | 776.8 | 154.4 | 285.8 | 430.6 | 257.9 | 218.8 | 286.2 | 140.3 | 318.5 |
| 13 | 0.0 | 257.13 | 141.4 | 0.0 | 57.4 | 85.0 | 0.0 | 164.3 | 242.7 | 0.0 | 205.4 | 155.4 | 0.0 | 18.6 | 227.5 |
| 14 | 0.0 | 250.8 | 120.1 | 0.0 | 47.4 | 46.8 | 0.0 | 263.0 | 211.1 | 0.0 | 145.3 | 98.8 | 0.0 | 243.5 | 130.1 |
| 15 | 0.0 | 196.9 | 101.5 | 0.0 | 7.8 | 17.5 | 0.0 | 91.6 | 183.9 | 0.0 | 111.9 | 51.4 | 0.0 | 56.7 | 66.3 |
| 16 | 0.0 | 108.4 | 84.5 | 0.0 | 29.3 | 0.0 | 0.0 | 115.4 | 154.7 | 0.0 | 58.2 | 23.6 | 0.0 | 40.4 | 22.9 |
| 17 | 0.0 | 98.9 | 68.8 | 0.0 | 39.9 | 0.0 | 0.0 | 83.3 | 138.1 | 0.0 | 88.6 | 0.0 | 0.0 | 42.7 | 0.0 |
| 18 | 0.0 | 88.0 | 56.2 | 0.0 | 25.5 | 0.0 | 0.0 | 96.1 | 113.2 | 0.0 | 34.9 | 0.0 | 0.0 | 12.6 | 0.0 |
| 19 | 0.0 | 50.5 | 44.6 | 0.0 | 10.4 | 0.0 | 0.0 | 97.6 | 84.4 | 0.0 | 29.7 | 0.0 | 0.0 | 53.1 | 0.0 |
| 20 | 0.0 | 80.1 | 30.2 | 0.0 | 4.7 | 0.0 | 0.0 | 227.7 | 83.2 | 0.0 | 26.2 | 0.0 | 0.0 | 9.4 | 0.0 |
| 21 | 0.0 | 34.9 | 16.8 | 0.0 | 5.5 | 0.0 | 0.0 | 68.1 | 55.0 | 0.0 | 25.5 | 0.0 | 0.0 | 4.0 | 0.0 |
| 22 | 0.0 | 22.8 | 0.0 | 0.0 | 9.4 | 0.0 | 0.0 | 84.8 | 32.6 | 0.0 | 5.5 | 0.0 | 0.0 | 1.7 | 0.0 |
| 23 | 0.0 | 28.1 | 0.0 | 0.0 | 3.2 | 0.0 | 0.0 | 35.8 | 39.5 | 0.0 | | 0.0 | 0.0 | 2.9 | 0.0 |
| 24 | 0.0 | 25.5 | 0.0 | 0.0 | 4.0 | 0.0 | 0.0 | 38.1 | 0.0 | 0.0 | 9.1 | 0.0 | 0.0 | 8.9 | 0.0 |
| 25 | 0.0 | | | 0.0 | 0.2 | 0.0 | 0.0 | 23.2 | 0.0 | 0.0 | 4.7 | 0.0 | 0.0 | 2.4 | 0.0 |
| 26 | 0.0 | 16.5 | 0.0 | | | | | | | | | | | | |
| 27 | 0.0 | 12.4 | 0.0 | | | | | | | | | | | | |
| 28 | 0.0 | 24.1 | 0.0 | | | | | | | | | | | | |
| 29 | 0.0 | 26.3 | 0.0 | | | | | | | | | | | | |
| 30 | 0.0 | | | | | | | | | | | | | | |
| 31 | 0.0 | 10.7 | 0.0 | | | | | | | | | | | | |
| 32 | 0.0 | 14.0 | 0.0 | | | | | | | | | | | | |
| 33 | 0.0 | 23.1 | 0.0 | | | | | | | | | | | | |